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(54) **USE OF PROTEINS AND PEPTIDES ENCODED BY THE GENOME OF A NOVEL SARS-ASSOCIATED CORONAVIRUS STRAIN**

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C07K	*14/505*	(2006.01)
C07K	*16/46*	(2006.01)
C12Q	*1/70*	(2006.01)
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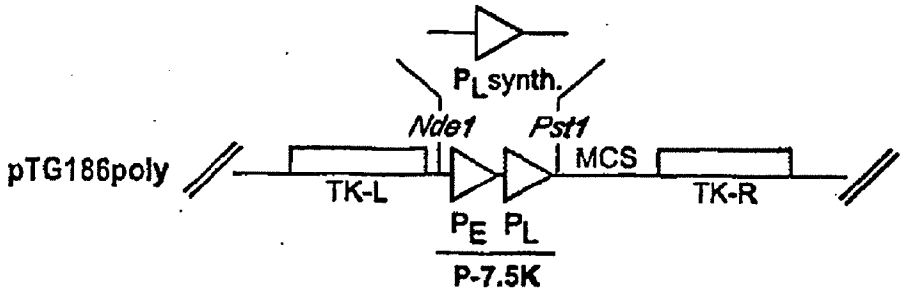
C07K	*16/08*	(2006.01)
C07H	*21/04*	(2006.01)
A61K	*35/76*	(2006.01)

(52) **U.S. Cl.** **424/186.1**; 424/93.2; 435/243; 435/320.1; 435/5; 514/44; 530/350; 530/388.3; 530/391.1; 536/23.72

(57) **ABSTRACT**

The invention relates to the use of proteins and peptides coded by the genome of the isolated or purified strain of severe acute respiratory syndrome (SARS)-associated coronavirus, resulting from sample reference number 031589 and, in particular, to the use of protein S and the derivative antibodies thereof as diagnostic reagents and as a vaccine.



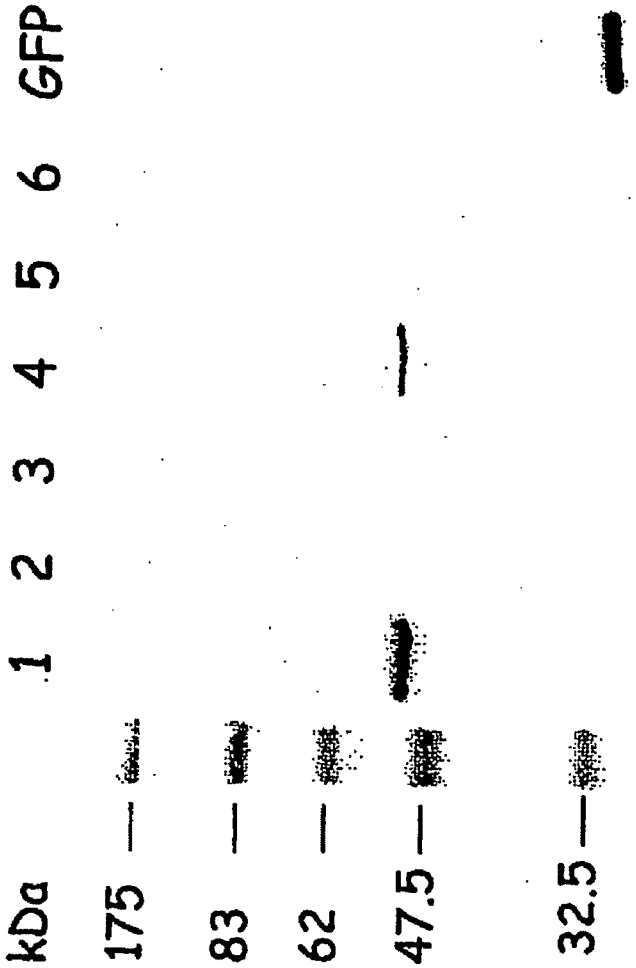


FIGURE 1

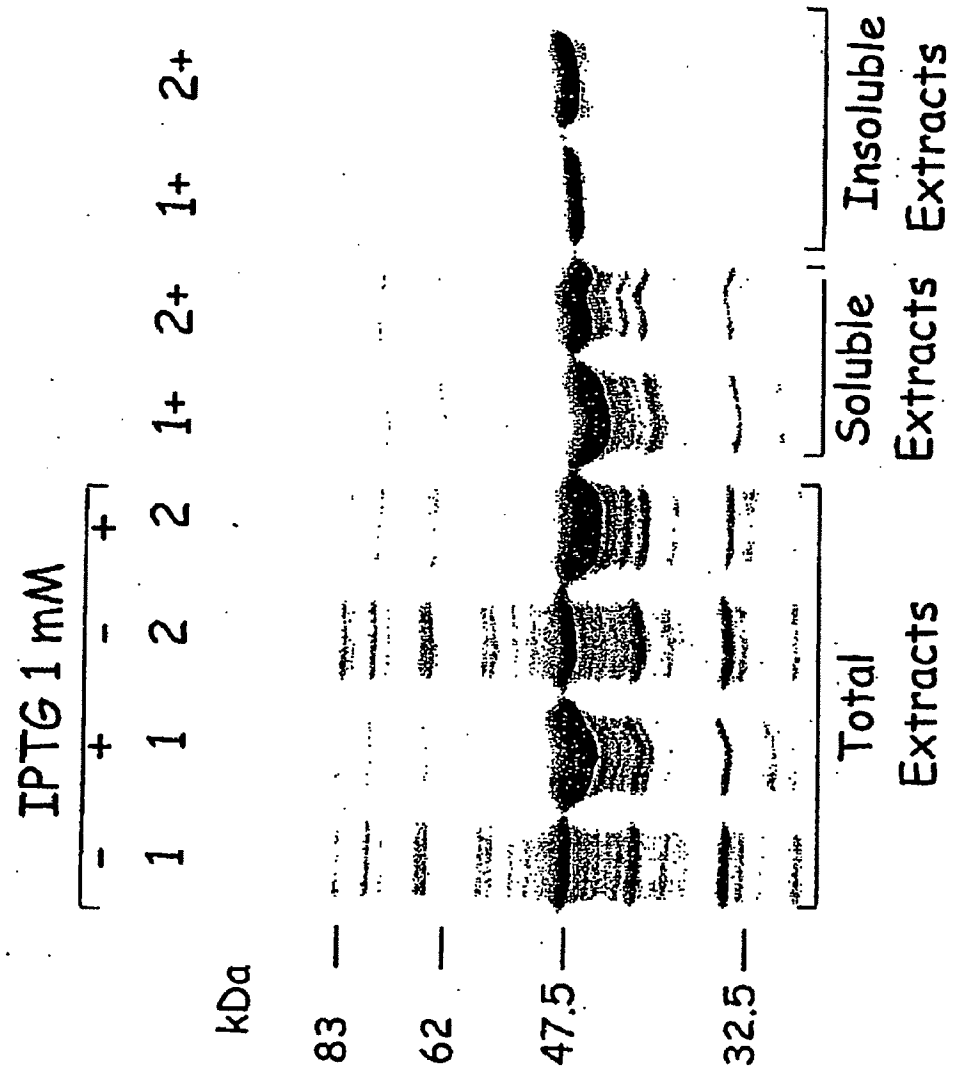

IPTG 1 mM
- + - +
1 1 2 2 1+ 2+ 1+ 2+
kDa
83
62
47.5
32.5
Total Extracts
Soluble Extracts
Insoluble Extracts

FIGURE 2

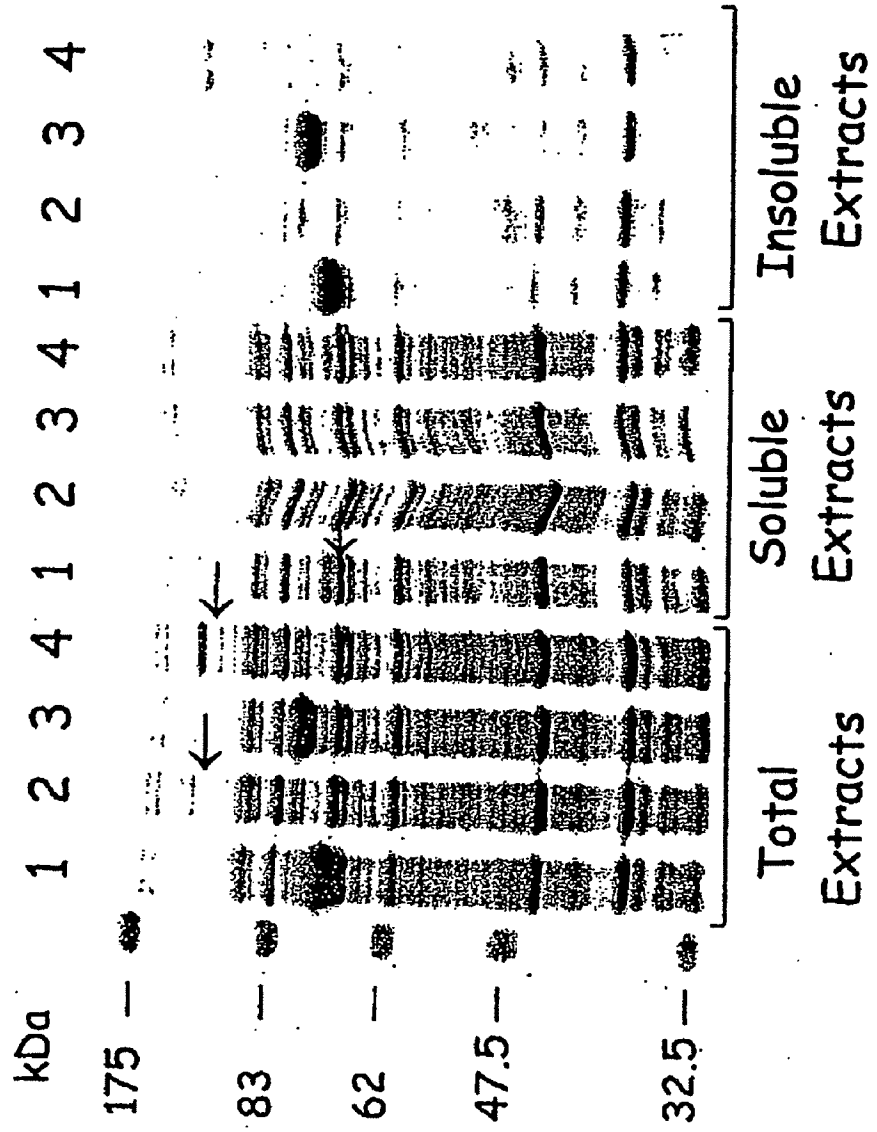

kDa
175
83
62
47.5
32.5
1 2 3 4
1 2 3 4
1 2 3 4
Total Extracts
Soluble Extracts
Insoluble Extracts

FIGURE 3

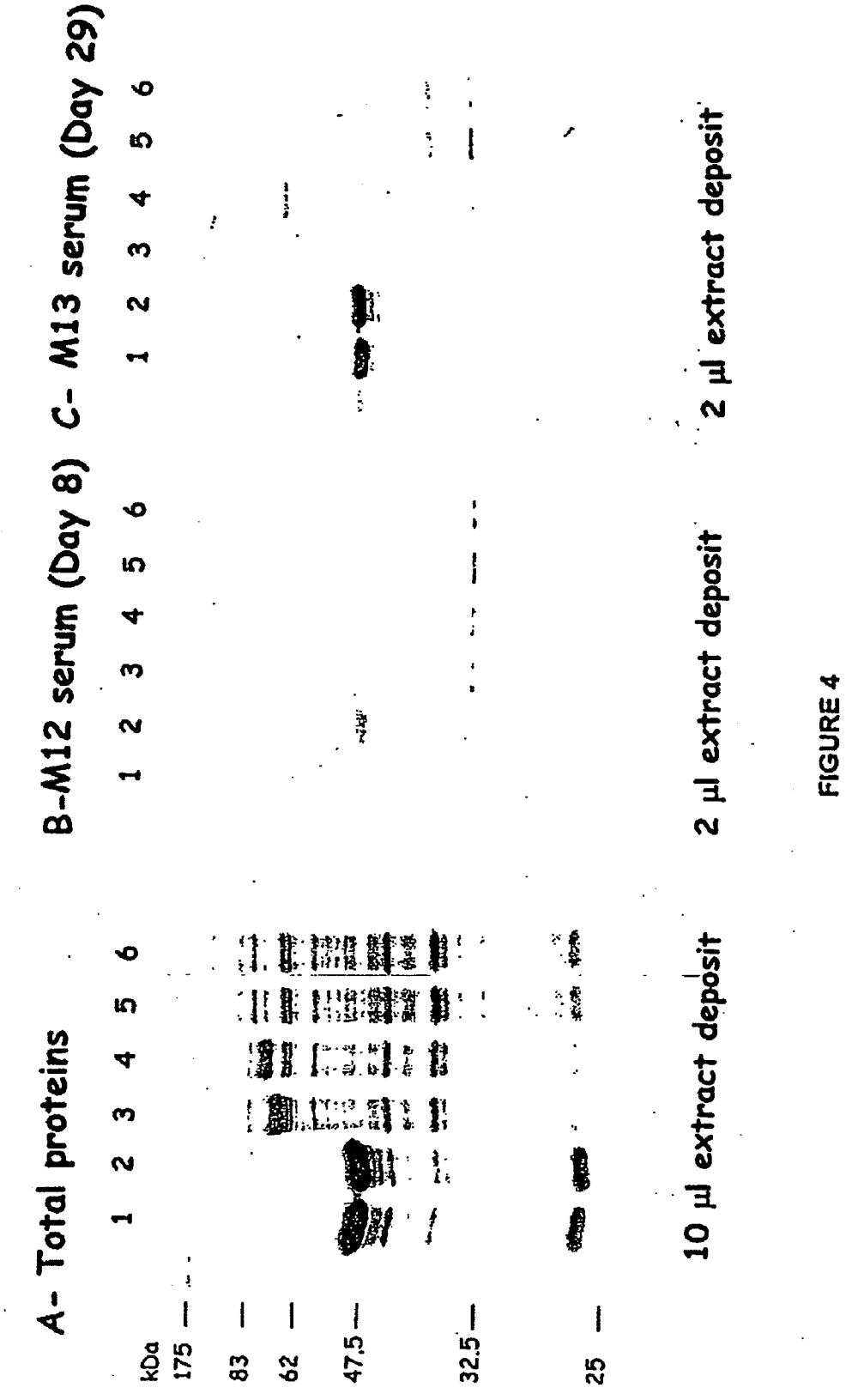

A- Total proteins
1 2 3 4 5 6
kDa
175
83
62
47.5
32.5
25
10 µl extract deposit
B-M12 serum (Day 8)
1 2 3 4 5 6
2 µl extract deposit
C- M13 serum (Day 29)
1 2 3 4 5 6
2 µl extract deposit

FIGURE 4

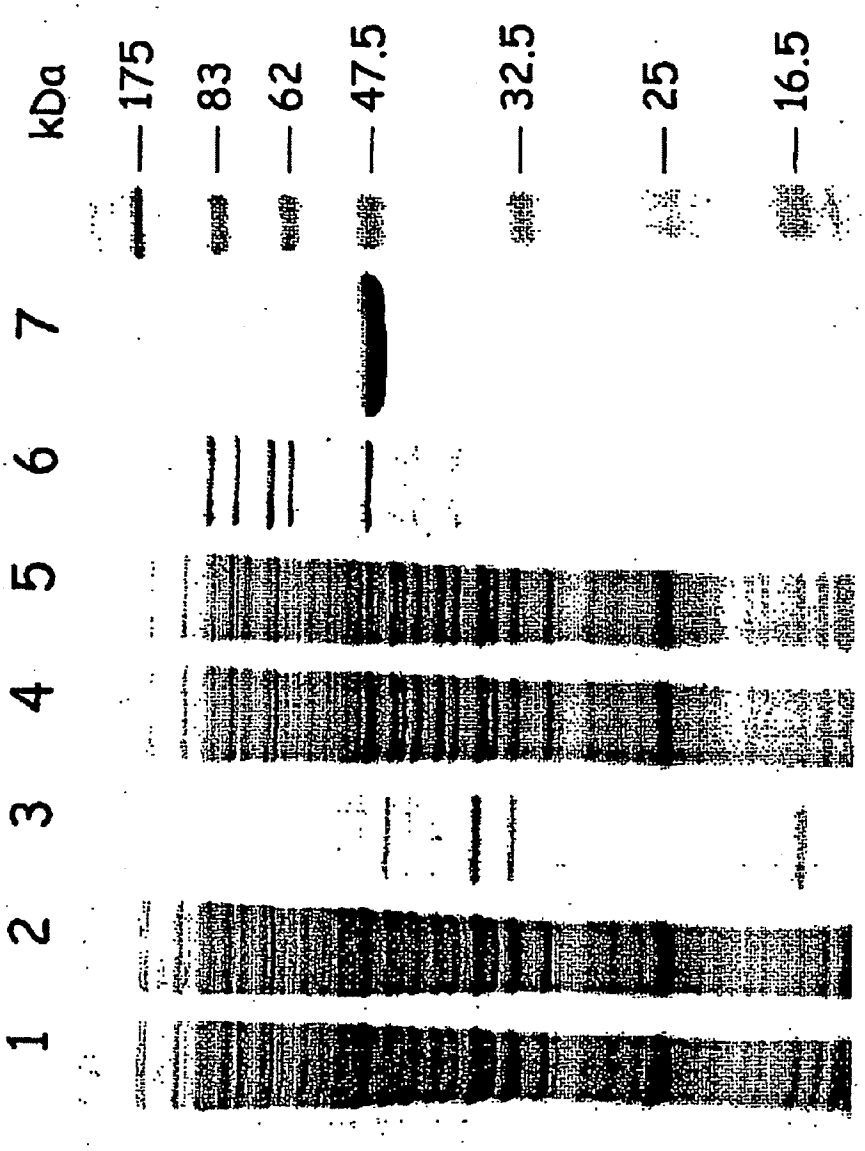


FIGURE 5

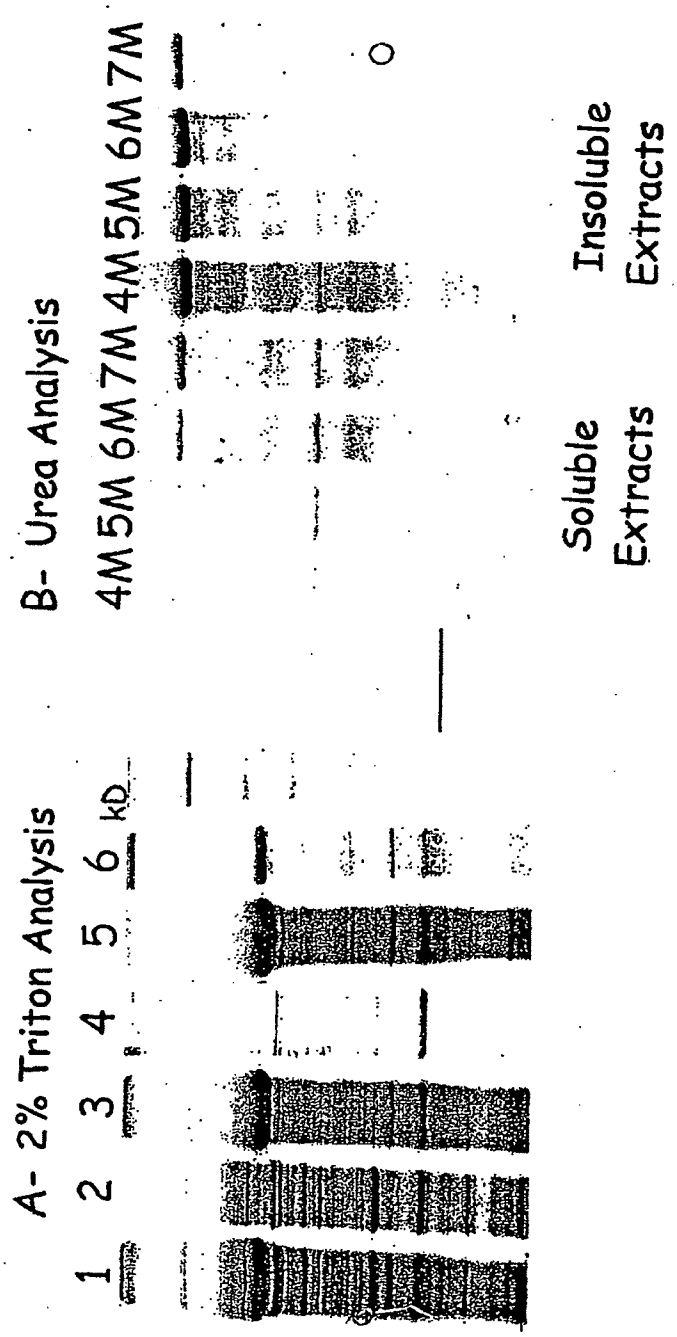

A- 2% Triton Analysis
1 2 3 4 5 6 kD
B- Urea Analysis
4M 5M 6M 7M 4M 5M 6M 7M
Soluble Extracts
Insoluble Extracts

FIGURE 6

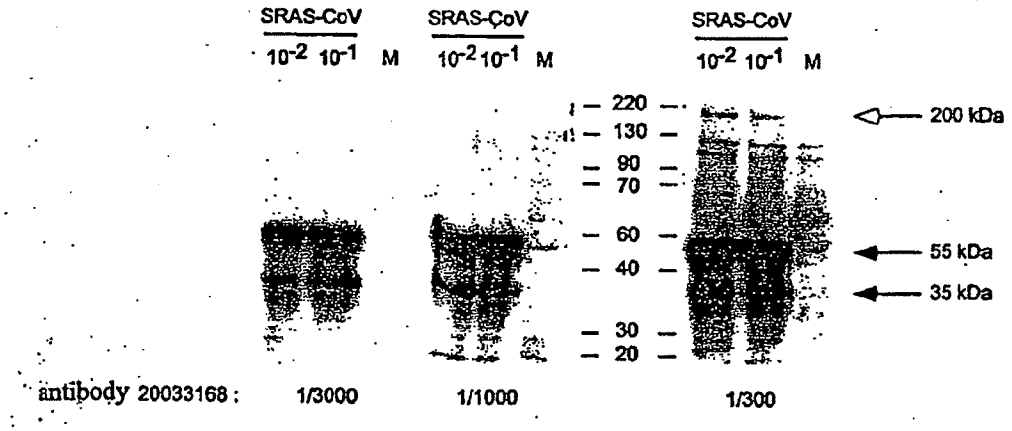

SRAS-CoV
10-2 10-1 M
SRAS-CoV
10-2 10-1 M
SRAS-CoV
10-2 10-1 M
220
130
90
70
60
40
30
20
200 kDa
55 kDa
35 kDa
antibody 20033168 :
1/3000
1/1000
1/300

FIGURE 7

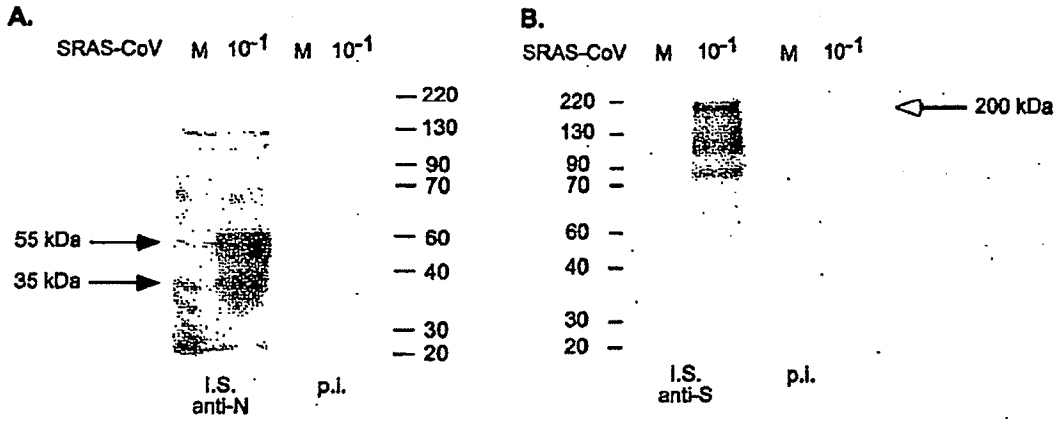
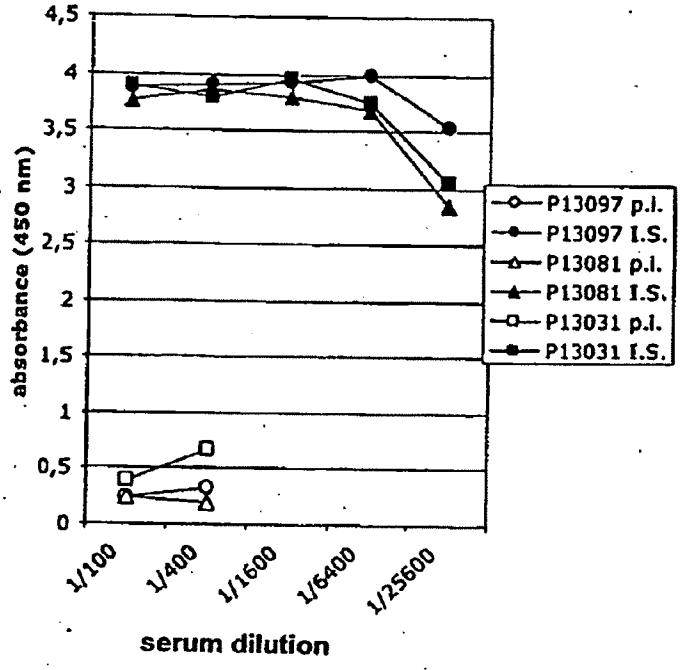

A.
SRAS-CoV
M
10-1
M
10-1
220
130
90
70
60
40
30
20
55 kDa
35 kDa
I.S.
anti-N
p.i.
B.
SRAS-CoV
M
10-1
M
10-1
220
130
90
70
60
40
30
20
200 kDa
I.S.
anti-S
p.i.

FIGURE 8

A


4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
absorbance (450 nm)
1/100
1/400
1/1600
1/6400
1/25600
serum dilution
P13097 p.i.
P13097 I.S.
P13081 p.i.
P13081 I.S.
P13031 p.i.
P13031 I.S.

B

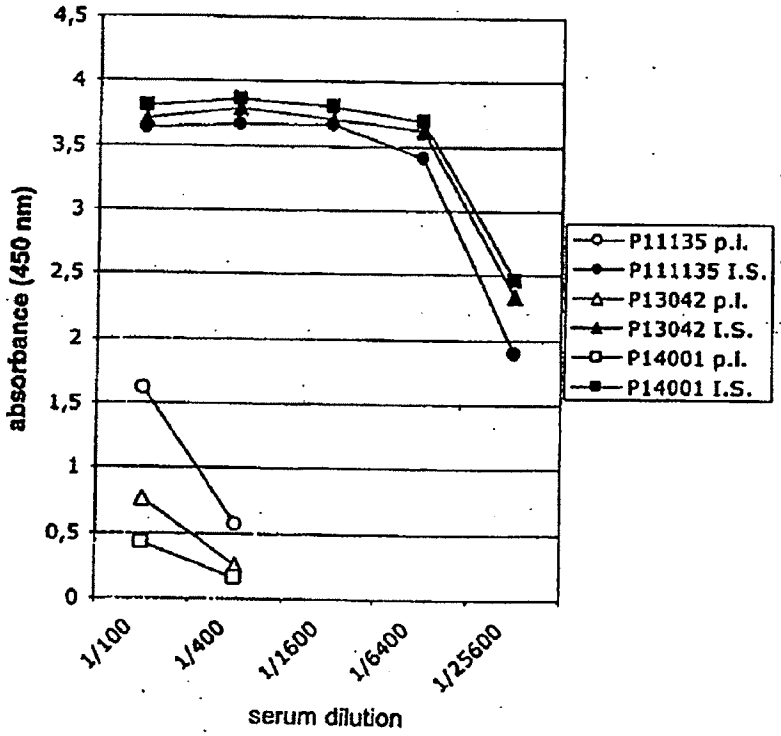
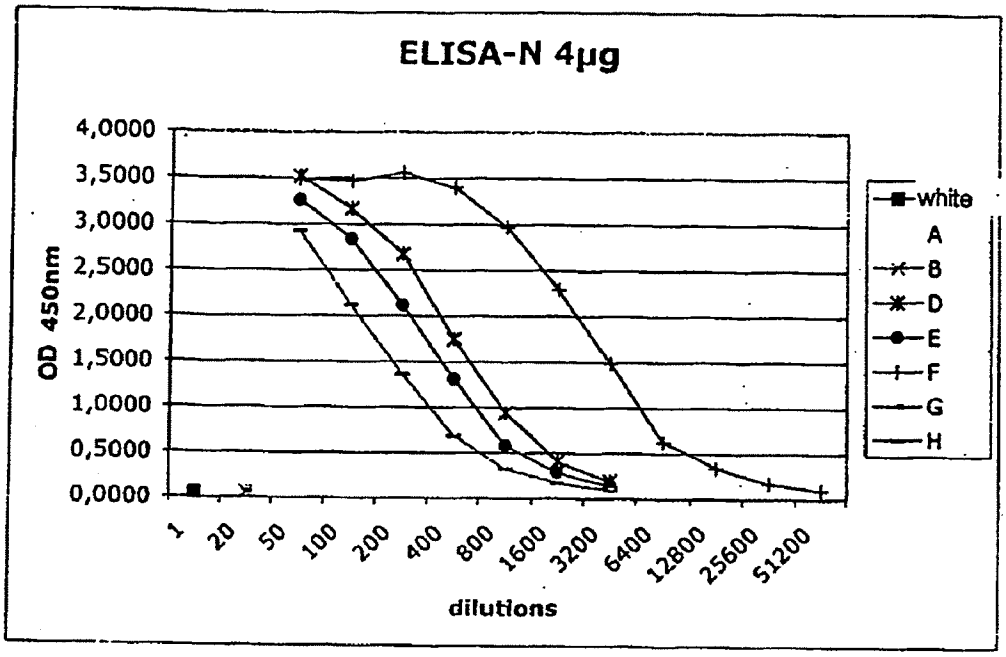

4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
absorbance (450 nm)
1/100
1/400
1/1600
1/6400
1/25600
serum dilution
P11135 p.i.
P111135 I.S.
P13042 p.i.
P13042 I.S.
P14001 p.i.
P14001 I.S.

FIGURE 9


ELISA-N 4µg
OD 450nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

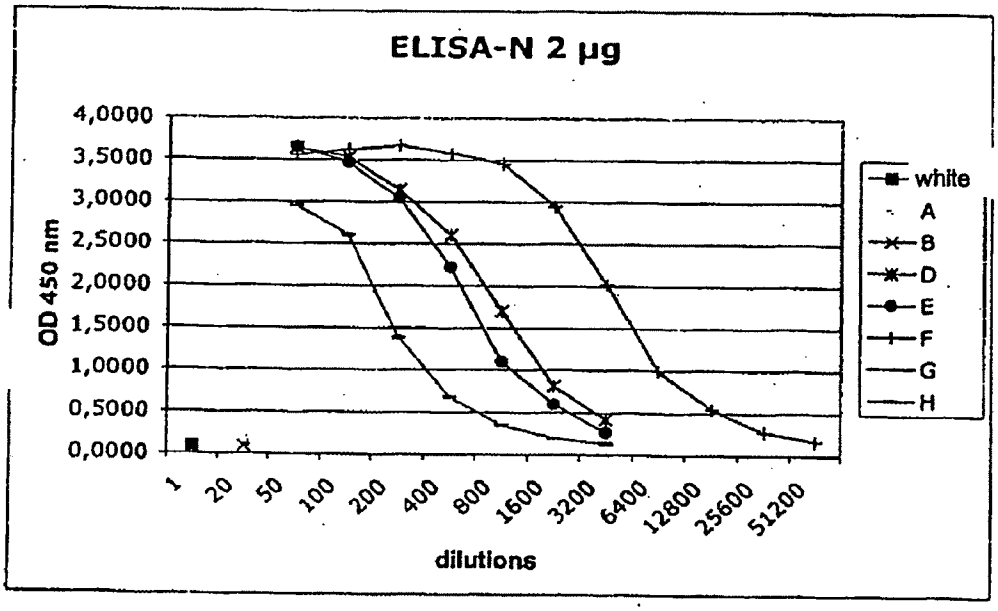

ELISA-N 2 µg
OD 450 nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

FIGURE 10a

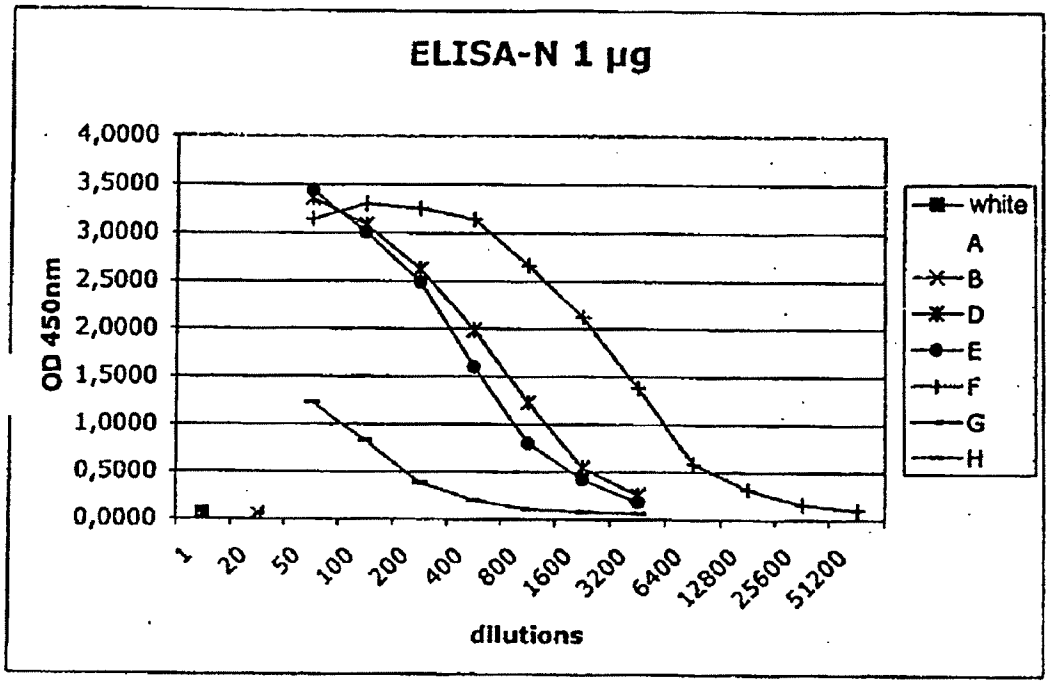

ELISA-N 1 µg
OD 450nm
4,0000
3,5000
3,0000
2,5000
2,0000
1,5000
1,0000
0,5000
0,0000
1
20
50
100
200
400
800
1600
3200
6400
12800
25600
51200
dilutions
white
A
B
D
E
F
G
H

FIGURE 10b

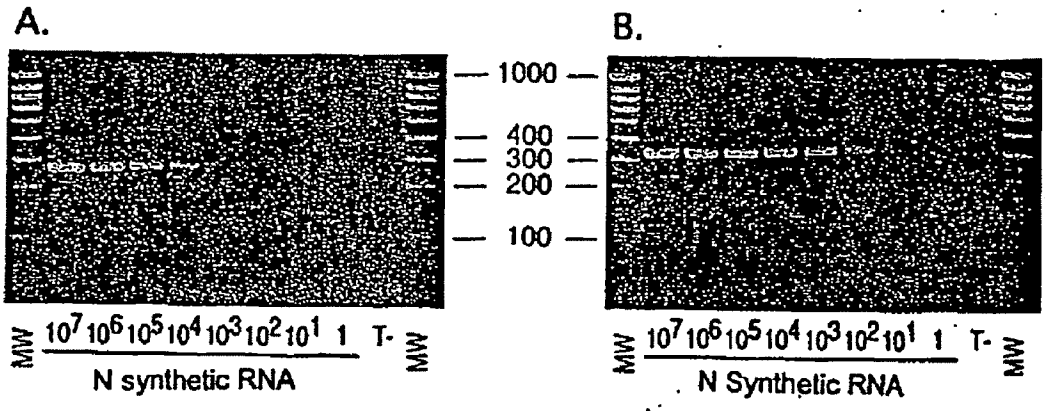

A.
B.
1000
400
300
200
100
MW
10^7 10^6 10^5 10^4 10^3 10^2 10^1 1 T-
MW
N synthetic RNA
MW
10^7 10^6 10^5 10^4 10^3 10^2 10^1 1 T-
MW
N Synthetic RNA

FIGURE 11

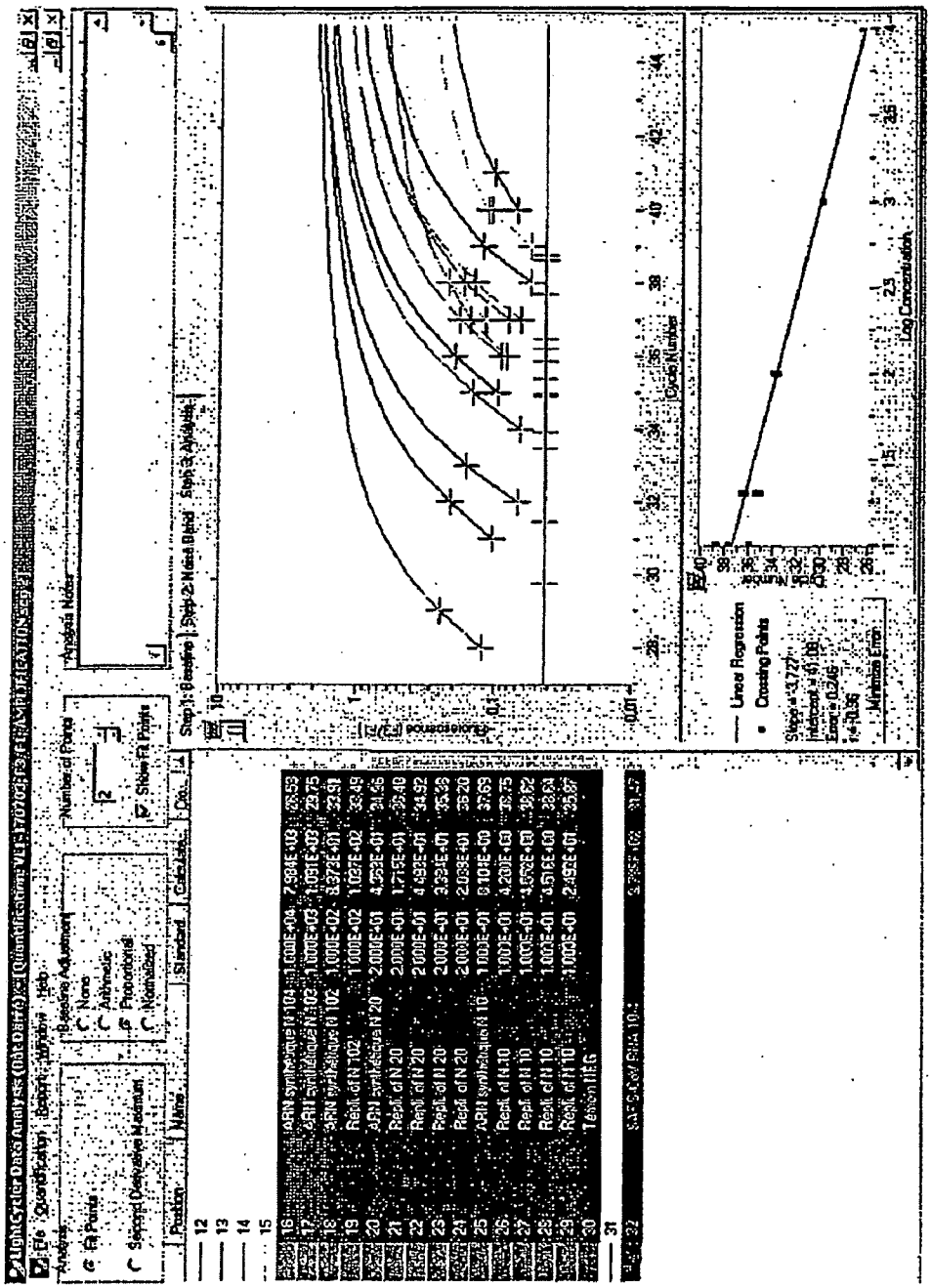


FIGURE 12

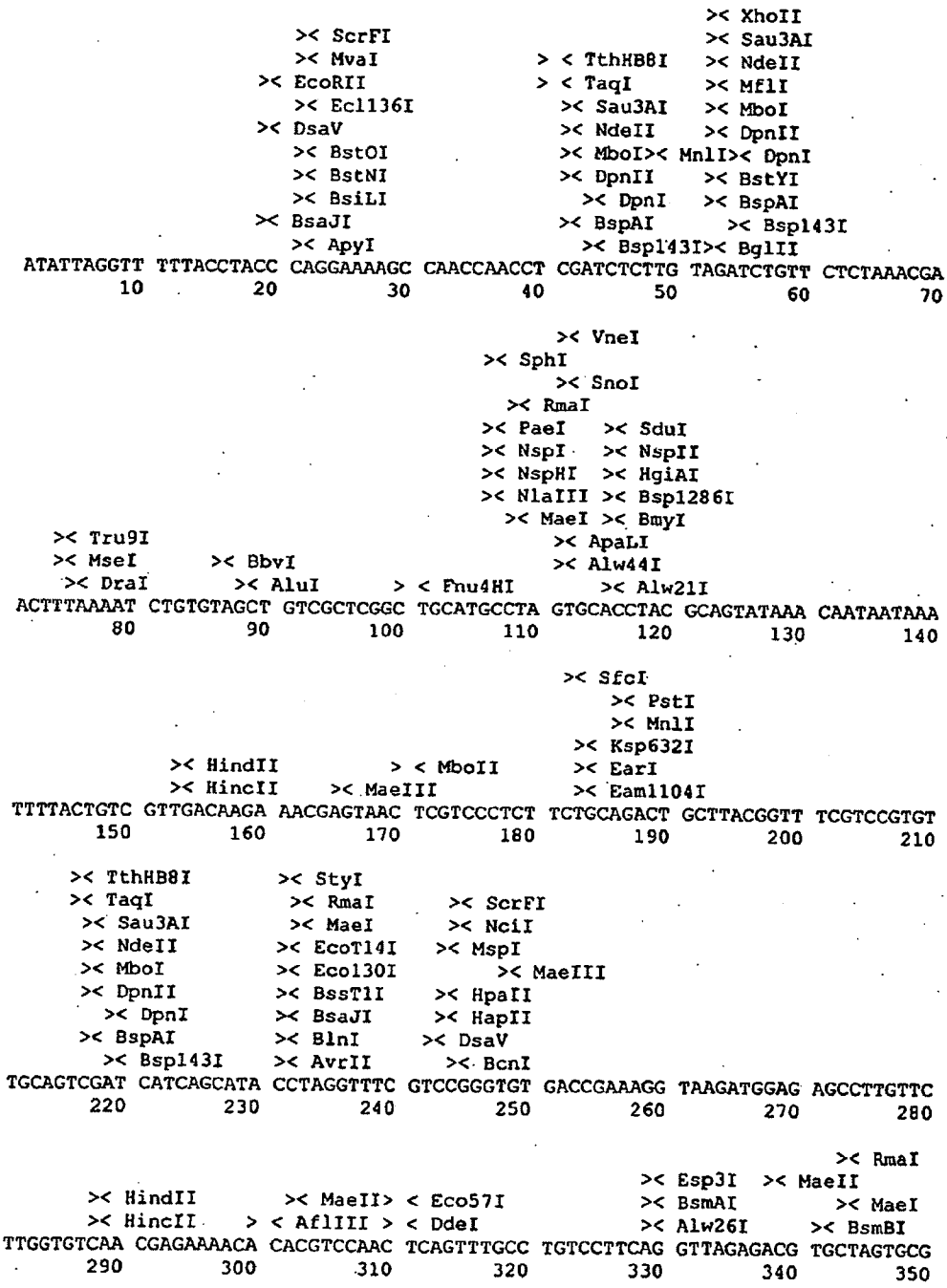

>< XhoII
>< ScrFI >< Sau3AI
>< MvaI > < TthHB8I >< NdeII
>< EcoRII > < TaqI >< MfeI
>< Ecl136I >< Sau3AI >< MboI
>< DsaV >< NdeII >< DpnII
>< BstOI >< MboI>< MnlI>< DpnI
>< BstNI >< DpnII >< BstYI
>< BsiLI >< DpnI >< BspAI
>< BsaJI >< BspAI >< Bsp143I
>< ApyI >< Bsp143I>< BglII
ATATTAGGTT TTTACCTACC CAGGAAAAGC CAACCAACCT CGATCTCTTG TAGATCTGTT CTCTAAACGA
10 20 30 40 50 60 70
>< VneI
>< SphI
>< SnoI
>< RmaI
>< PaeI >< SduI
>< NspI >< NspII
>< NspHI >< HgiAI
>< NlaIII >< Bsp1286I
>< MaeI >< BmyI
>< Tru9I >< ApaLI
>< MseI >< BbvI >< Alw44I
>< DraI >< AluI > < Fnu4HI >< Alw21I
ACTTTAAAAT CTGTGTAGCT GTCGCTCGGC TGCATGCCTA GTGCACCTAC GCAGTATAAA CAATAATAAA
80 90 100 110 120 130 140
>< SfcI
>< PstI
>< MnlI
>< Ksp632I
>< HindII > < MboII >< EarI
>< HincII >< MaeIII >< Eam1104I
TTTTACTGTC GTTGACAAGA AACGAGTAAC TCGTCCCTCT TCTGCAGACT GCTTACGGTT TCGTCCGTGT
150 160 170 180 190 200 210
>< TthHB8I >< StyI
>< TaqI >< RmaI >< ScrFI
>< Sau3AI >< MaeI >< NciI
>< NdeII >< EcoT14I >< MspI
>< MboI >< Eco130I >< MaeIII
>< DpnII >< BssT1I >< HpaII
>< DpnI >< BsaJI >< HapII
>< BspAI >< BlnI >< DsaV
>< Bsp143I >< AvrII >< BcnI
TGCAGTCGAT CATCAGCATA CCTAGGTTTC GTCCGGGTGT GACCGAAAGG TAAGATGGAG AGCCTTGTTC
220 230 240 250 260 270 280
>< RmaI
>< Esp3I >< MaeII
>< HindII >< MaeII> < Eco57I >< BsmAI >< MaeI
>< HincII > < AflIII > < DdeI >< Alw26I >< BsmBI
TTGGTGTCAA CGAGAAAACA CACGTCCAAC TCAGTTTGCC TGTCCTTCAG GTTAGAGACG TGCTAGTGCG
290 300 310 320 330 340 350

FIGURE 13.1

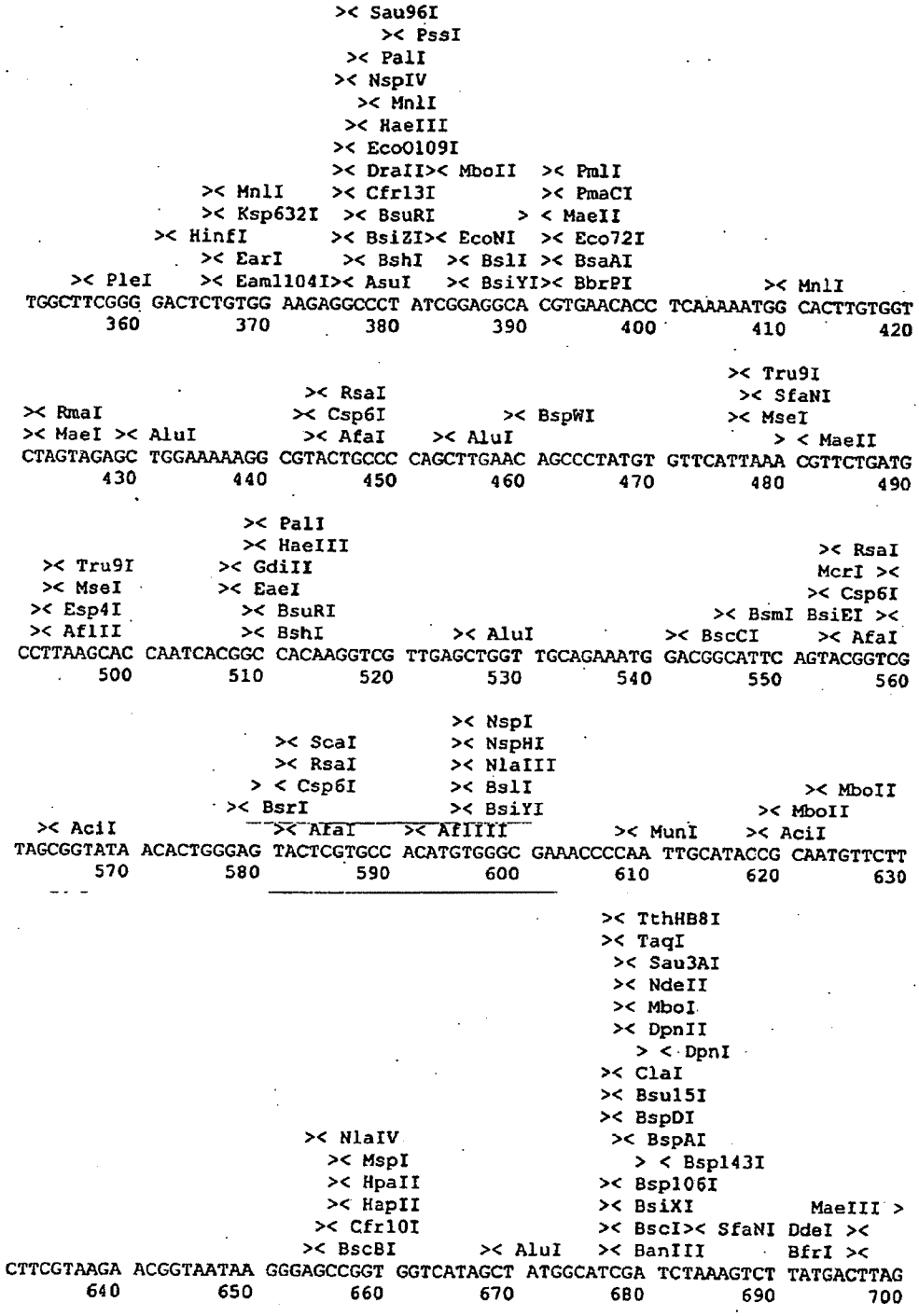

>< Sau96I
>< PssI
>< PalI
>< NspIV
>< MnlI
>< HaeIII
>< Eco0109I
>< DraII>< MboII >< PmlI
>< MnlI >< Cfr13I >< PmaCI
>< Ksp632I >< BsuRI > < MaeII
>< HinfI >< BsiZI>< EcoNI >< Eco72I
>< EarI >< BshI >< BslI >< BsaAI
>< PleI >< Eam1104I>< AsuI >< BsiYI>< BbrPI >< MnlI
TGGCTTCGGG GACTCTGTGG AAGAGGCCCT ATCGGAGGCA CGTGAACACC TCAAAAATGG CACTTGTGGT
360 370 380 390 400 410 420
>< Tru9I
>< SfaNI
>< RsaI >< MseI
>< RmaI >< Csp6I >< BspWI
>< MaeI >< AluI >< AfaI >< AluI > < MaeII
CTAGTAGAGC TGGAAAAAGG CGTACTGCCC CAGCTTGAAC AGCCCTATGT GTTCATTAAA CGTTCTGATG
430 440 450 460 470 480 490
>< PalI
>< HaeIII
>< RsaI
>< Tru9I >< GdiII McrI ><
>< MseI >< EaeI >< Csp6I
>< Esp4I >< BsuRI >< BsmI BsiEI ><
>< AflII >< BshI >< AluI >< BscCI >< AfaI
CCTTAAGCAC CAATCACGGC CACAAGGTCG TTGAGCTGGT TGCAGAAATG GACGGCATTC AGTACGGTCG
500 510 520 530 540 550 560
>< NspI
>< ScaI >< NspHI
>< RsaI >< NlaIII
> < Csp6I >< BslI >< MboII
>< BsrI >< BsiYI >< MboII
>< AciI >< AfaI >< AflIII >< MunI >< AciI
TAGCGGTATA ACACTGGGAG TACTCGTGCC ACATGTGGGC GAAACCCCAA TTGCATACCG CAATGTTCTT
570 580 590 600 610 620 630
>< TthHB8I
>< TaqI
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
> < DpnI
>< ClaI
>< Bsu15I
>< BspDI
>< NlaIV >< BspAI
>< MspI > < Bsp143I
>< HpaII >< Bsp106I
>< HapII >< BsiXI MaeIII >
>< Cfr10I >< BscI>< SfaNI DdeI ><
>< BscBI >< AluI >< BanIII BfrI ><
CTTCGTAAGA ACGGTAATAA GGGAGCCGGT GGTCATAGCT ATGGCATCGA TCTAAAGTCT TATGACTTAG
640 650 660 670 680 690 700

FIGURE 13.2

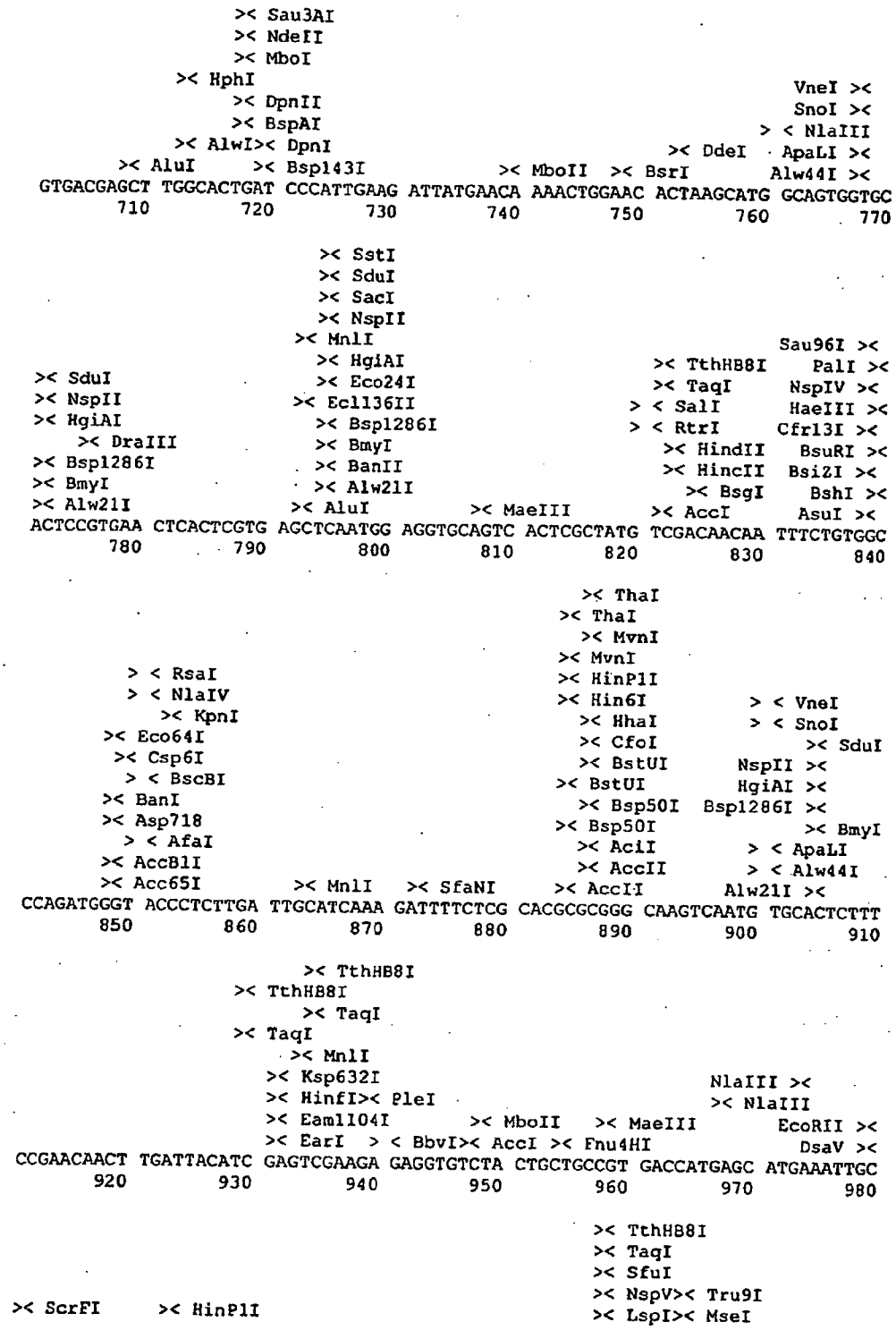

>< Sau3AI
>< NdeII
>< MboI
>< HphI
>< DpnII
>< BspAI
>< AlwI>< DpnI
>< AluI >< Bsp143I >< MboII >< BsrI
>< DdeI
VneI ><
SnoI ><
> < NlaIII
ApaLI ><
Alw44I ><
GTGACGAGCT TGGCACTGAT CCCATTGAAG ATTATGAACA AAACTGGAAC ACTAAGCATG GCAGTGGTGC
710 720 730 740 750 760 770
>< SstI
>< SduI
>< SacI
>< NspII
>< MnlI
>< HgiAI
>< Eco24I
>< Ecl136II
>< Bsp1286I
>< BmyI
>< BanII
>< Alw21I
>< AluI >< MaeIII
>< TthHB8I
>< TaqI
> < SalI
> < RtrI
>< HindII
>< HincII
>< BsgI
>< AccI
Sau96I ><
PalI ><
NspIV ><
HaeIII ><
Cfr13I ><
BsuRI ><
Bsi2I ><
BshI ><
AsuI ><
>< SduI
>< NspII
>< HgiAI
>< DraIII
>< Bsp1286I
>< BmyI
>< Alw21I
ACTCCGTGAA CTCACTCGTG AGCTCAATGG AGGTGCAGTC ACTCGCTATG TCGACAACAA TTTCTGTGGC
780 790 800 810 820 830 840
>< ThaI
>< ThaI
>< MvnI
>< MvnI
>< HinP1I
>< Hin6I
>< HhaI
>< CfoI
>< BstUI
>< BstUI
>< Bsp50I
>< Bsp50I
>< AciI
>< AccII
> < RsaI
> < NlaIV
>< KpnI
>< Eco64I
>< Csp6I
> < BscBI
>< BanI
>< Asp718
> < AfaI
>< Acc65I
>< AccB1I
>< MnlI >< SfaNI >< AccII
> < VneI
> < SnoI
>< SduI
NspII ><
HgiAI ><
Bsp1286I ><
>< BmyI
> < ApaLI
> < Alw44I
Alw21I ><
CCAGATGGGT ACCCTCTTGA TTGCATCAAA GATTTTCTCG CACGCGCGGG CAAGTCAATG TGCACTCTTT
850 860 870 880 890 900 910
>< TthHB8I
>< TthHB8I
>< TaqI
>< TaqI
>< MnlI
>< Ksp632I
>< HinfI>< PleI
>< Eam1104I >< MboII >< MaeIII
>< EarI > < BbvI>< AccI >< Fnu4HI
NlaIII ><
>< NlaIII
EcoRII ><
DsaV ><
CCGAACAACT TGATTACATC GAGTCGAAGA GAGGTGTCTA CTGCTGCCGT GACCATGAGC ATGAAATTGC
920 930 940 950 960 970 980
>< TthHB8I
>< TaqI
>< SfuI
>< NspV>< Tru9I
>< LspI>< MseI
>< ScrFI >< HinP1I

FIGURE 13.3

```
><MvaI    ><Hin6I              ><SduI    ><Csp45I
><Ecl136I   ><HhaI             ><NspII   ><BstBI
><BstOI     ><HaeII            ><HgiAI   ><Bsp119I
><BstNI   ><Eco47III           ><Bsp1286I><BsiCI
><BsiLI     ><CfoI             ><BmyI    ><Bpu14I
><ApyI ><DdeI ><Bsp143II ><AluI  ><Alw21I   ><AsuII
CTGGTTCACT GAGCGCTCTG ATAAGAGCTA CGAGCACCAG ACACCCTTCG AAATTAAGAG TGCCAAGAAA
      990       1000       1010       1020       1030       1040       1050

                                                 ><Tru9I
                                                 ><MseI
                          ><BsmI
                       ><BscCI                        > < MnlI
TTTGACACTT TCAAAGGGGA ATGCCCAAAG TTTGTGTTTC CTCTTAACTC AAAAGTCAAA GTCATTCAAC
     1060       1070       1080       1090       1100       1110       1120

  ><PmlI
  ><PmaCI
 ><MaeII
  ><Eco72I
  ><BsaAI                          ><NlaIII            ><RsaI
  ><BbrPI                                    ><Bst1107I ><Csp6I
 ><AflIII     ><MnlI><DdeI                  ><AccI      ><AfaI
CACGTGTTGA AAAGAAAAAG ACTGAGGGTT TCATGGGGCG TATACGCTCT GTGTACCCTG TTGCATCTCC
     1130       1140       1150       1160       1170       1180       1190

 ><SfaNI
      ><MaeIII          ><AccI                              NlaIII><
ACAGGAGTGT AACAATATGC ACTTGTCTAC CTTGATGAAA TGTAATCATT GCGATGAAGT TTCATGGCAG
     1200       1210       1220       1230       1240       1250       1260

                                                            ><SinI
                                                            ><Sau96I
                                                           PssI ><
                                                             ><Psp5II
                                                            ><PpuMI
                                                            ><NspIV
                                                             ><NspHII
                                                            ><Eco47I
                                                            ><DraII
                                                            ><Cfr13I
                                                            ><BsiZI
                                                            ><Bme18I
                                                            ><AvaII
                                                            ><AsuI
><MaeII                                                 Eco0109I ><AflIII >
ACGTGCGACT TTCTGAAAGC CACTTGTGAA CATTGTGGCA CTGAAAATTT AGTTATTGAA GGACCTACTA
     1270       1280       1290       1300       1310       1320       1330

                                                          Van91I ><
                                                               SinI ><
      ><RsaI                                                 Sau96I ><
  ><NspI                                                    PflMI ><
      ><NlaIV                                                 NspIV ><
  ><NlaIII                                                    NspHII >
  ><NspHI><KpnI                                             Eco47I ><
    ><Eco64I                                                Cfr13I ><
     ><Csp6I                                               BslI ><
      ><BscBI                                                 BsiZI ><
    ><BanI                                                 BsiYI ><
    ><Asp718                                                Bme18I ><
      ><AfaI                                                AvaII ><
    ><AccB1I                                                 AsuI ><
```

FIGURE 13. 4

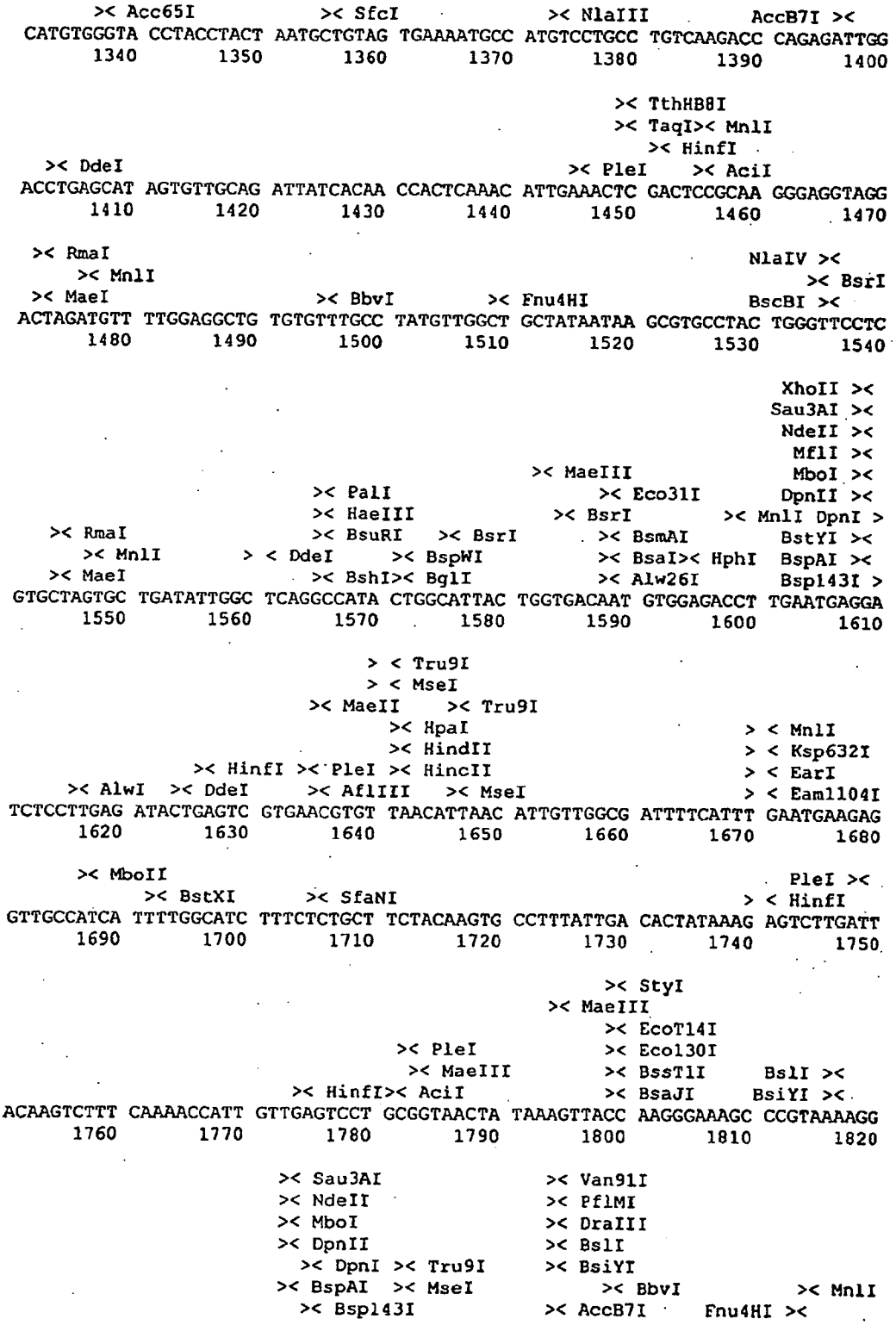

>< Acc65I
>< SfcI
>< NlaIII
AccB7I ><
CATGTGGGTA CCTACCTACT AATGCTGTAG TGAAAATGCC ATGTCCTGCC TGTCAAGACC CAGAGATTGG
1340 1350 1360 1370 1380 1390 1400
>< TthHB8I
>< TaqI>< MnlI
>< HinfI
>< DdeI
>< PleI
>< AciI
ACCTGAGCAT AGTGTTGCAG ATTATCACAA CCACTCAAAC ATTGAAACTC GACTCCGCAA GGGAGGTAGG
1410 1420 1430 1440 1450 1460 1470
>< RmaI
NlaIV ><
>< MnlI
>< BsrI
>< MaeI
>< BbvI
>< Fnu4HI
BscBI ><
ACTAGATGTT TTGGAGGCTG TGTGTTTGCC TATGTTGGCT GCTATAATAA GCGTGCCTAC TGGGTTCCTC
1480 1490 1500 1510 1520 1530 1540
XhoII ><
Sau3AI ><
NdeII ><
MflI ><
>< MaeIII
MboI ><
>< PalI
>< Eco31I
DpnII ><
>< HaeIII
>< BsrI
>< MnlI DpnI >
>< RmaI
>< BsuRI
>< BsrI
>< BsmAI
BstYI ><
>< MnlI
> < DdeI
>< BspWI
>< BsaI>< HphI
BspAI ><
>< MaeI
>< BshI>< BglI
>< Alw26I
BspI43I >
GTGCTAGTGC TGATATTGGC TCAGGCCATA CTGGCATTAC TGGTGACAAT GTGGAGACCT TGAATGAGGA
1550 1560 1570 1580 1590 1600 1610
> < Tru9I
> < MseI
>< MaeII
>< Tru9I
>< HpaI
> < MnlI
>< HindII
> < Ksp632I
>< HinfI
>< PleI
>< HincII
> < EarI
>< AlwI
>< DdeI
>< AflIII
>< MseI
> < Eam1104I
TCTCCTTGAG ATACTGAGTC GTGAACGTGT TAACATTAAC ATTGTTGGCG ATTTTCATTT GAATGAAGAG
1620 1630 1640 1650 1660 1670 1680
>< MboII
PleI ><
>< BstXI
>< SfaNI
> < HinfI
GTTGCCATCA TTTTGGCATC TTTCTCTGCT TCTACAAGTG CCTTTATTGA CACTATAAAG AGTCTTGATT
1690 1700 1710 1720 1730 1740 1750
>< StyI
>< MaeIII
>< EcoT14I
>< PleI
>< Eco130I
>< MaeIII
>< BssT1I
BslI ><
>< HinfI>< AciI
>< BsaJI
BsiYI ><
ACAAGTCTTT CAAAACCATT GTTGAGTCCT GCGGTAACTA TAAAGTTACC AAGGGAAAGC CCGTAAAAGG
1760 1770 1780 1790 1800 1810 1820
>< Sau3AI
>< Van91I
>< NdeII
>< PflMI
>< MboI
>< DraIII
>< DpnII
>< BslI
>< DpnI >< Tru9I
>< BsiYI
>< BspAI >< MseI
>< BbvI
>< MnlI
>< Bsp143I
>< AccB7I
Fnu4HI ><

FIGURE 13.5

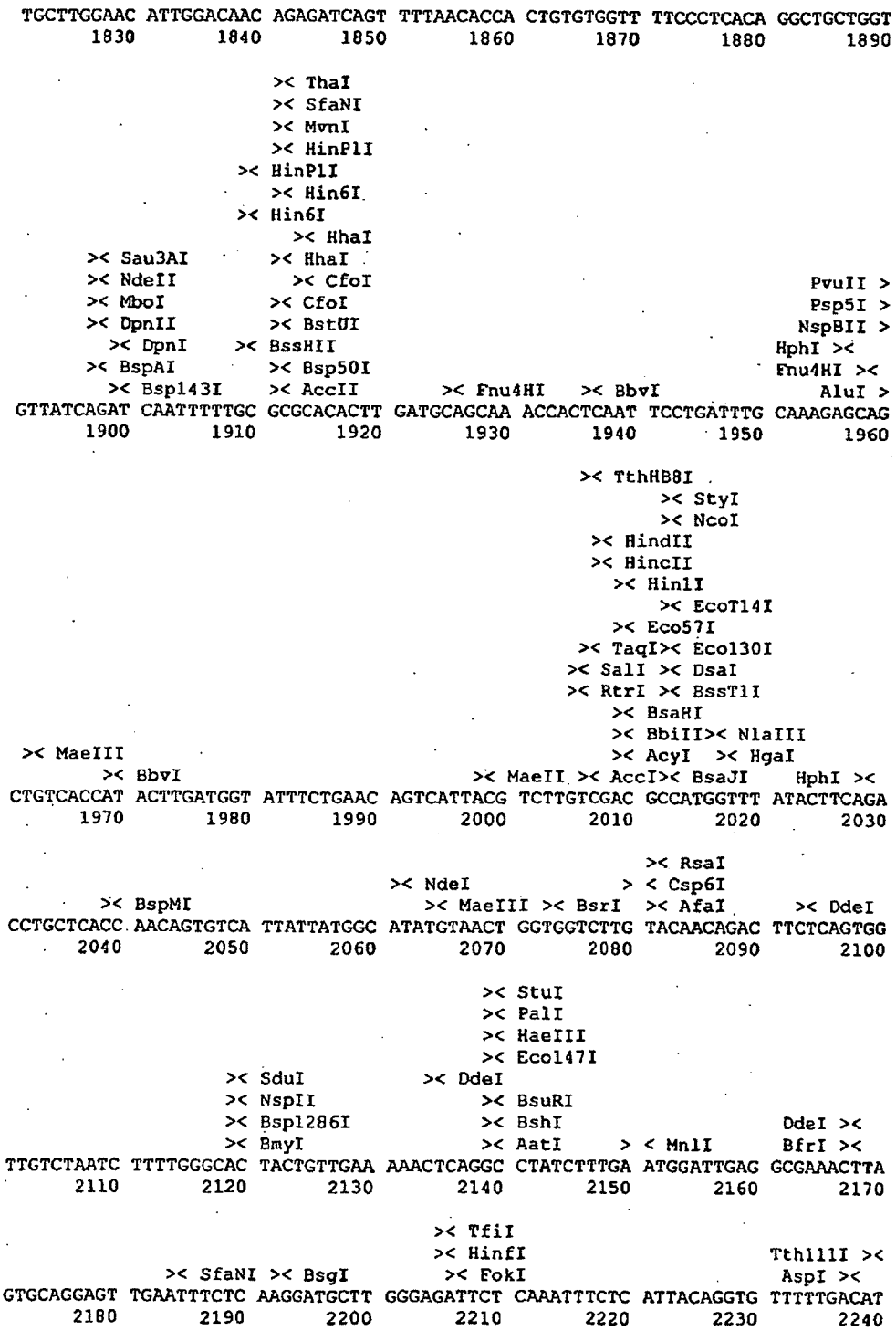

TGCTTGGAAC ATTGGACAAC AGAGATCAGT TTTAACACCA CTGTGTGGTT TTCCCTCACA GGCTGCTGGT
1830 1840 1850 1860 1870 1880 1890
>< ThaI
>< SfaNI
>< MvnI
>< HinP1I
>< HinPlI
>< Hin6I
>< Hin6I
>< HhaI
>< Sau3AI
>< HhaI
>< NdeII
>< CfoI
PvuII >
>< MboI
>< CfoI
Psp5I >
>< DpnII
>< BstUI
NspBII >
>< DpnI
>< BssHII
HphI ><
>< BspAI
>< Bsp50I
Fnu4HI ><
>< Bsp143I
>< AccII
>< Fnu4HI
>< BbvI
AluI >
GTTATCAGAT CAATTTTTGC GCGCACACTT GATGCAGCAA ACCACTCAAT TCCTGATTTG CAAAGAGCAG
1900 1910 1920 1930 1940 1950 1960
>< TthHB8I
>< StyI
>< NcoI
>< HindII
>< HincII
>< HinlI
>< EcoT14I
>< Eco57I
>< TaqI>< Eco130I
>< SalI >< DsaI
>< RtrI >< BssT1I
>< BsaHI
>< BbiII>< NlaIII
>< MaeIII
>< AcyI >< HgaI
>< BbvI
>< MaeII >< AccI>< BsaJI
HphI ><
CTGTCACCAT ACTTGATGGT ATTTCTGAAC AGTCATTACG TCTTGTCGAC GCCATGGTTT ATACTTCAGA
1970 1980 1990 2000 2010 2020 2030
>< RsaI
>< NdeI
> < Csp6I
>< BspMI
>< MaeIII >< BsrI
>< AfaI
>< DdeI
CCTGCTCACC AACAGTGTCA TTATTATGGC ATATGTAACT GGTGGTCTTG TACAACAGAC TTCTCAGTGG
2040 2050 2060 2070 2080 2090 2100
>< StuI
>< PalI
>< HaeIII
>< Eco147I
>< SduI
>< DdeI
>< NspII
>< BsuRI
>< Bspl286I
>< BshI
DdeI ><
>< BmyI
>< AatI
> < MnlI
BfrI ><
TTGTCTAATC TTTTGGGCAC TACTGTTGAA AAACTCAGGC CTATCTTTGA ATGGATTGAG GCGAAACTTA
2110 2120 2130 2140 2150 2160 2170
>< TfiI
>< HinfI
Tth111I ><
>< SfaNI >< BsgI
>< FokI
AspI ><
GTGCAGGAGT TGAATTTCTC AAGGATGCTT GGGAGATTCT CAAATTTCTC ATTACAGGTG TTTTTGACAT
2180 2190 2200 2210 2220 2230 2240

FIGURE 13.6

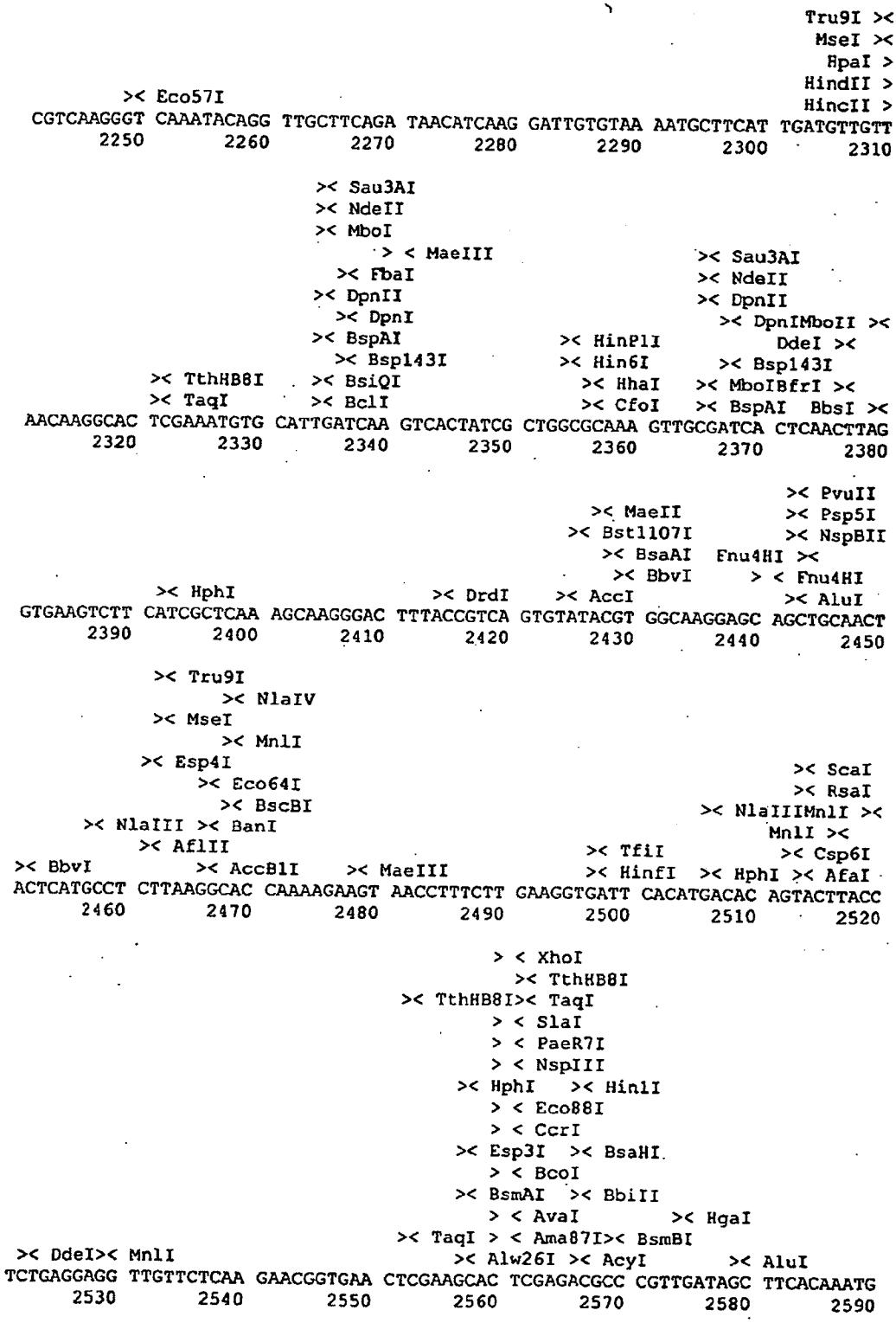
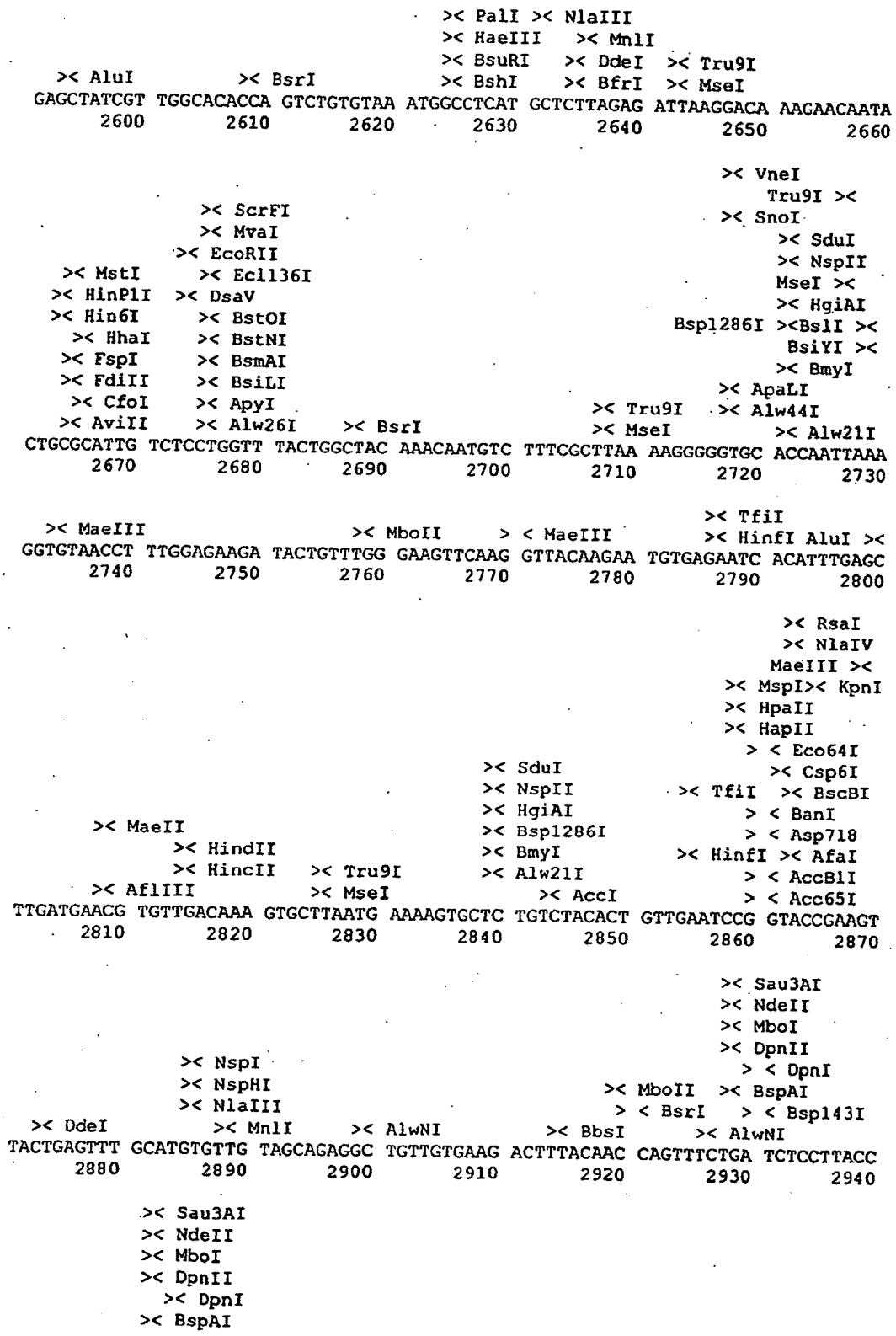

Tru9I ><
MseI ><
HpaI >
HindII >
HincII >
>< Eco57I
CGTCAAGGGT CAAATACAGG TTGCTTCAGA TAACATCAAG GATTGTGTAA AATGCTTCAT TGATGTTGTT
2250 2260 2270 2280 2290 2300 2310
>< Sau3AI
>< NdeII
>< MboI
> < MaeIII
>< Sau3AI
>< FbaI
>< NdeII
>< DpnII
>< DpnII
>< DpnI
>< DpnIMboII ><
DdeI ><
>< BspAI
>< HinP1I
>< Bsp143I
>< Bsp143I
>< Hin6I
>< TthHB8I
>< BsiQI
>< HhaI
>< MboIBfrI ><
>< TaqI
>< BclI
>< CfoI
>< BspAI BbsI ><
AACAAGGCAC TCGAAATGTG CATTGATCAA GTCACTATCG CTGGCGCAAA GTTGCGATCA CTCAACTTAG
2320 2330 2340 2350 2360 2370 2380
>< PvuII
>< MaeII
>< Psp5I
>< Bst1107I
>< NspBII
>< BsaAI
Fnu4HI ><
>< BbvI
> < Fnu4HI
>< HphI
>< DrdI
>< AccI
>< AluI
GTGAAGTCTT CATCGCTCAA AGCAAGGGAC TTTACCGTCA GTGTATACGT GGCAAGGAGC AGCTGCAACT
2390 2400 2410 2420 2430 2440 2450
>< Tru9I
>< NlaIV
>< MseI
>< MnlI
>< Esp4I
>< ScaI
>< Eco64I
>< RsaI
>< BscBI
>< NlaIIIMnlI ><
>< NlaIII >< BanI
MnlI ><
>< AflII
>< TfiI
>< Csp6I
>< BbvI
>< AccBlI
>< MaeIII
>< HinfI
>< HphI >< AfaI
ACTCATGCCT CTTAAGGCAC CAAAAGAAGT AACCTTTCTT GAAGGTGATT CACATGACAC AGTACTTACC
2460 2470 2480 2490 2500 2510 2520
> < XhoI
>< TthHB8I
>< TthHB8I>< TaqI
> < SlaI
> < PaeR7I
> < NspIII
>< HphI >< HinlI
> < Eco88I
> < CcrI
>< Esp3I >< BsaHI
> < BcoI
>< BsmAI >< BbiII
> < AvaI
>< HgaI
>< TaqI > < Ama87I>< BsmBI
>< DdeI>< MnlI
>< Alw26I >< AcyI
>< AluI
TCTGAGGAGG TTGTTCTCAA GAACGGTGAA CTCGAAGCAC TCGAGACGCC CGTTGATAGC TTCACAAATG
2530 2540 2550 2560 2570 2580 2590

FIGURE 13.7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>< HaeIII >< MnlI
>< BsuRI >< DdeI >< Tru9I
>< AluI >< BsrI >< BshI >< BfrI >< MseI
GAGCTATCGT TGGCACACCA GTCTGTGTAA ATGGCCTCAT GCTCTTAGAG ATTAAGGACA AAGAACAATA
2600 2610 2620 2630 2640 2650 2660
>< VneI
Tru9I ><
>< SnoI
>< SduI
>< NspII
MseI ><
>< HgiAI
Bsp1286I ><BslI ><
BsiYI ><
>< BmyI
>< ApaLI
>< ScrFI
>< MvaI
>< EcoRII
>< MstI >< Ecl136I
>< HinP1I >< DsaV
>< Hin6I >< BstOI
>< HhaI >< BstNI
>< FspI >< BsmAI
>< FdiII >< BsiLI
>< CfoI >< ApyI
>< AviII >< Alw26I >< BsrI >< Tru9I >< Alw44I
>< MseI >< Alw21I
CTGCGCATTG TCTCCTGGTT TACTGGCTAC AAACAATGTC TTTCGCTTAA AAGGGGGTGC ACCAATTAAA
2670 2680 2690 2700 2710 2720 2730
>< TfiI
>< MaeIII >< MboII > < MaeIII >< HinfI AluI ><
GGTGTAACCT TTGGAGAAGA TACTGTTTGG GAAGTTCAAG GTTACAAGAA TGTGAGAATC ACATTTGAGC
2740 2750 2760 2770 2780 2790 2800
>< RsaI
>< NlaIV
MaeIII ><
>< MspI>< KpnI
>< HpaII
>< HapII
> < Eco64I
>< Csp6I
>< SduI
>< NspII >< TfiI >< BscBI
>< HgiAI > < BanI
>< MaeII >< Bsp1286I > < Asp718
>< HindII >< BmyI >< HinfI >< AfaI
>< HincII >< Tru9I >< Alw21I > < AccBlI
>< AflIII >< MseI >< AccI > < Acc65I
TTGATGAACG TGTTGACAAA GTGCTTAATG AAAAGTGCTC TGTCTACACT GTTGAATCCG GTACCGAAGT
2810 2820 2830 2840 2850 2860 2870
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
> < DpnI
>< NspI
>< NspHI
>< NlaIII
>< MboII >< BspAI
> < BsrI > < Bsp143I
>< DdeI >< MnlI >< AlwNI >< BbsI >< AlwNI
TACTGAGTTT GCATGTGTTG TAGCAGAGGC TGTTGTGAAG ACTTTACAAC CAGTTTCTGA TCTCCTTACC
2880 2890 2900 2910 2920 2930 2940
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< BspAI

FIGURE 13.8

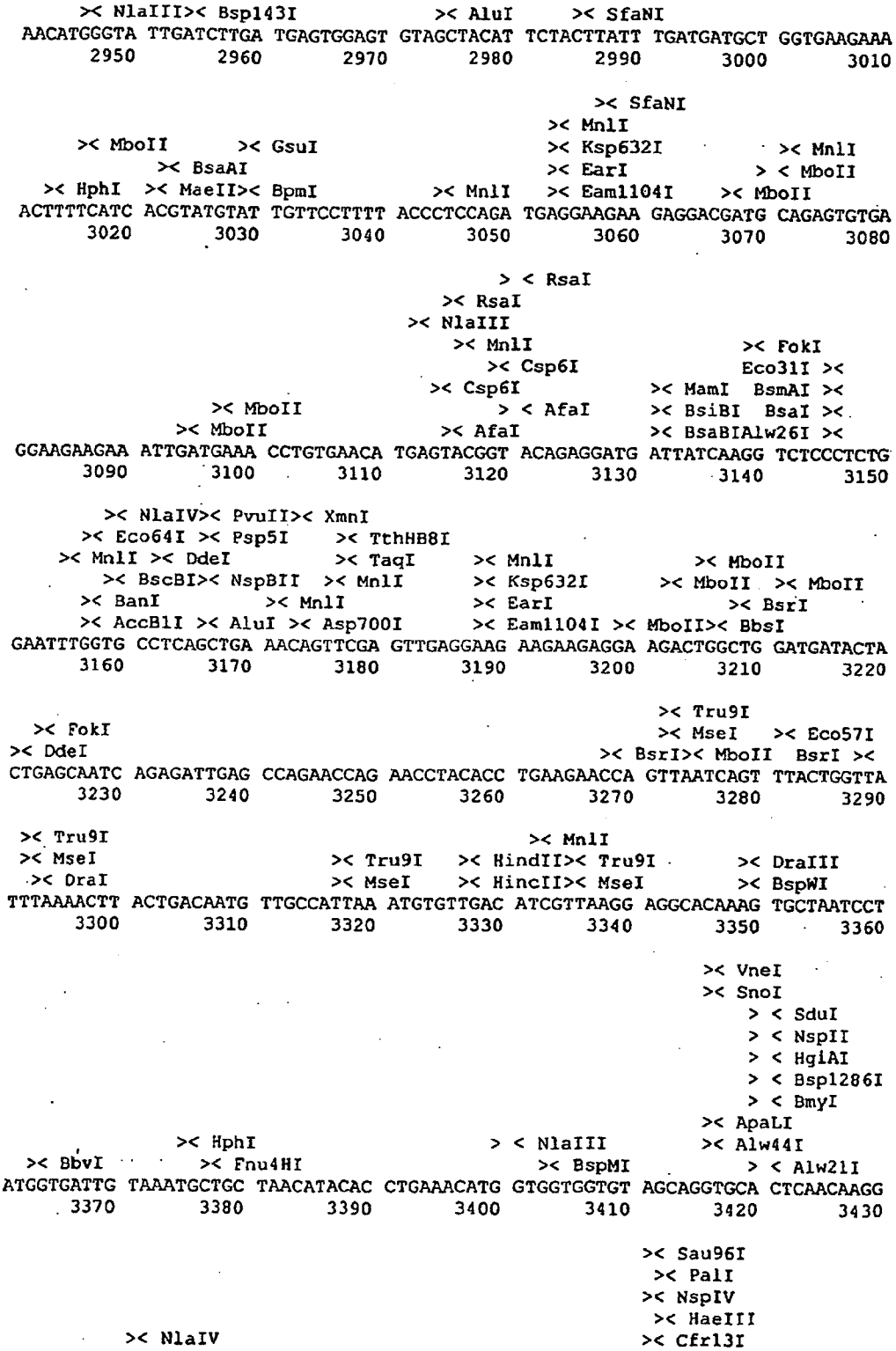

>< NlaIII>< Bsp143I >< AluI >< SfaNI
AACATGGGTA TTGATCTTGA TGAGTGGAGT GTAGCTACAT TCTACTTATT TGATGATGCT GGTGAAGAAA
2950 2960 2970 2980 2990 3000 3010
>< SfaNI
>< MnlI
>< MboII >< GsuI >< Ksp632I >< MnlI
>< BsaAI >< EarI > < MboII
>< HphI >< MaeII>< BpmI >< MnlI >< Eam1104I >< MboII
ACTTTTCATC ACGTATGTAT TGTTCCTTTT ACCCTCCAGA TGAGGAAGAA GAGGACGATG CAGAGTGTGA
3020 3030 3040 3050 3060 3070 3080
> < RsaI
>< RsaI
>< NlaIII
>< MnlI >< FokI
>< Csp6I Eco31I ><
>< Csp6I >< MamI BsmAI ><
>< MboII > < AfaI >< BsiBI BsaI ><
>< MboII >< AfaI >< BsaBIAlw26I ><
GGAAGAAGAA ATTGATGAAA CCTGTGAACA TGAGTACGGT ACAGAGGATG ATTATCAAGG TCTCCCTCTG
3090 3100 3110 3120 3130 3140 3150
>< NlaIV>< PvuII>< XmnI
>< Eco64I >< Psp5I >< TthHB8I
>< MnlI >< DdeI >< TaqI >< MnlI >< MboII
>< BscBI>< NspBII >< MnlI >< Ksp632I >< MboII >< MboII
>< BanI >< MnlI >< EarI >< BsrI
>< AccB1I >< AluI >< Asp700I >< Eam1104I >< MboII>< BbsI
GAATTTGGTG CCTCAGCTGA AACAGTTCGA GTTGAGGAAG AAGAAGAGGA AGACTGGCTG GATGATACTA
3160 3170 3180 3190 3200 3210 3220
>< Tru9I
>< FokI >< MseI >< Eco57I
>< DdeI >< BsrI>< MboII BsrI ><
CTGAGCAATC AGAGATTGAG CCAGAACCAG AACCTACACC TGAAGAACCA GTTAATCAGT TTACTGGTTA
3230 3240 3250 3260 3270 3280 3290
>< Tru9I >< MnlI
>< MseI >< Tru9I >< HindII>< Tru9I >< DraIII
>< DraI >< MseI >< HincII>< MseI >< BspWI
TTTAAAACTT ACTGACAATG TTGCCATTAA ATGTGTTGAC ATCGTTAAGG AGGCACAAAG TGCTAATCCT
3300 3310 3320 3330 3340 3350 3360
>< VneI
>< SnoI
> < SduI
> < NspII
> < HgiAI
> < Bsp1286I
> < BmyI
>< ApaLI
>< HphI > < NlaIII >< Alw44I
>< BbvI >< Fnu4HI >< BspMI > < Alw21I
ATGGTGATTG TAAATGCTGC TAACATACAC CTGAAACATG GTGGTGGTGT AGCAGGTGCA CTCAACAAGG
3370 3380 3390 3400 3410 3420 3430
>< Sau96I
>< PalI
>< NspIV
>< HaeIII
>< NlaIV >< Cfr13I

FIGURE 13.9

```
        >< Eco64I                                                    >< BsuRI
           >< BscBI                                  > < Tru9I     >< BsiZI
        >< BanI                                      > < MseI      >< BshI      >< MnlI
        >< AccB1I>< NlaIII                                 >< AluI >< AsuI >< MnlI
CAACCAATGG TGCCATGCAA AAGGAGAGTG ATGATTACAT TAAGCTAAAT GGCCCTCTTA CAGTAGGAGG
      3440       3450       3460       3470       3480       3490       3500

                                                             >< SinI
                                                             >< Sau96I
                                                             >< NspIV
                                                          >< NspHI>< NspHII
                                                             >< Eco47I
                                                             >< Cfr13I
                                                          >< NlaIII    >< BspMI
                                                             >< BsiZI
                                                             >< Bme18I
                                                             >< AvaII MnlI ><
                                  > < DdeI                >< NspI>< AsuI FokI ><
GTCTTGTTTG CTTTCTGGAC ATAATCTTGC TAAGAAGTGT CTGCATGTTG TTGGACCTAA CCTAAATGCA
      3510       3520       3530       3540       3550       3560       3570

                    > < Tru9I
                 >< HphI> < MseI
                       >< Esp4I
                  >< AluI          > < NdeI
                       >< AflII>< Fnu4HI    >< BbvI
GGTGAGGACA TCCAGCTTCT TAAGGCAGCA TATGAAAATT TCAATTCACA GGACATCTTA CTTGCACCAT
      3580       3590       3600       3610       3620       3630       3640

                                                                      RsaI ><
                                                                    Csp6I ><
                     >< Eco57I                        >< BcgI          AfaI ><
TGTTGTCAGC AGGCATATTT GGTGCTAAAC CACTTCAGTC TTTACAAGTG TGCGTGCAGA CGGTTCGTAC
      3650       3660       3670       3680       3690       3700       3710

    >< BsgI                        >< BspMI
       >< BcgI/a                >< AluI                    >< NlaIII
ACAGGTTTAT ATTGCAGTCA ATGACAAAGC TCTTTATGAG CAGGTTGTCA TGGATTATCT TGATAACCTG
      3720       3730       3740       3750       3760       3770       3780

                                                          >< MnlI
    >< RmaI          > < MnlI       >< NlaIV          >< TfiI    >< MboII
    >< MaeI          >< Eco57I      >< BscBI          >< HinfI   >< DdeI
AAGCCTAGAG TGGAAGCACC TAAACAAGAG GAGCCACCAA ACACAGAAGA TTCCAAAACT GAGGAGAAAT
      3790       3800       3810       3820       3830       3840       3850

                                    >< Tru9I
                                          >< StuI
                                          >< PalI
                                    >< MseI   >< MnlI     >< MaeIII
                                          >< HaeIII       >< Eco0651
                                          >< Eco147I      >< Eco91I
     >< RsaI                              >< BsuRI              BstXI ><
    >< Csp6I       >< TthHB8I             >< BshI         >< BstPI
     >< AfaI       >< TaqI                >< AatI         >< BstEII
CTGTCGTACA GAAGCCTGTC GATGTGAAGC CAAAAATTAA GGCCTGCATT GATGAGGTTA CCACAACACT
      3860       3870       3880       3890       3900       3910       3920

                                                                    TfiI ><
                                                                  NlaIII ><
                                                                   HinfI ><
      >< DdeI                                        >< EcoRV   >< HindIII
```

FIGURE 13.10

```
  >< BsrI     >< MboII      >< MaeIII           >< Eco32I    >< AluI
GGAAGAAACT AAGTTTCTTA CCAATAAGTT ACTCTTGTTT GCTGATATCA ATGGTAAGCT TTACCATGAT
      3930       3940       3950       3960       3970       3980       3990

           >< NspI
           >< NspHI
           >< NlaIII              >< SfaNI
        >< MnlI                      > < EcoNI
            >< DdeI              >< MboII >< BslI          > < NlaIII
 >< DdeI    >< BfrI            >< HphI     >< BsiYI        >< FokI
TCTCAGAACA TGCTTAGAGG TGAAGATATG TCTTTCCTTG AGAAGGATGC ACCTTACATG GTAGGTGATG
      4000       4010       4020       4030       4040       4050       4060

     >< SpeI
      >< RmaI
      >< MaeI    >< EcoRV>< HphI                        >< SfaNI
     >< HphI     >< Eco32I                        >< MnlI         >< DdeI
TTATCACTAG TGGTGATATC ACTTGTGTTG TAATACCCTC CAAAAAGGCT GGTGGCACTA CTGAGATGCT
      4070       4080       4090       4100       4110       4120       4130

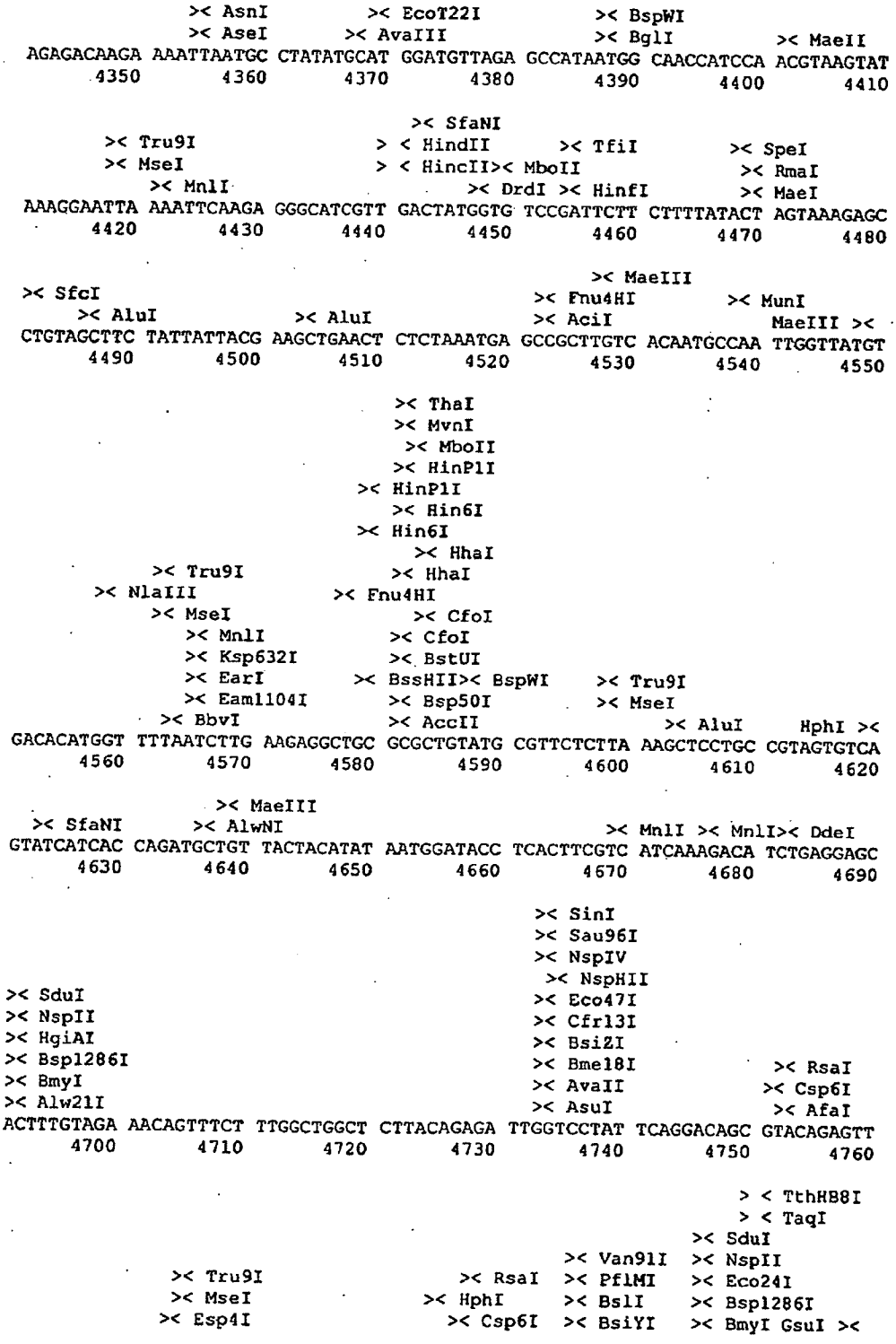
                                                    >< ScrFI
                                                  >< RsaI
                                                     >< MvaI
                                                  >< EcoRII
                                                     >< Ecl136I
                                                  >< DsaV
                                                >< Csp6I >< EcoNI
                                                     >< BstOI
                                                     >< BstNI
                                                     >< BsiLI
                                                   >< BsaJI
                                                >< BsaAI    >< BslI
                          >< MboII              >< MaeII>< ApyI
      >< AluI          >< BsrI                    >< AfaI   >< BsiYI
CTCAAGAGCT TTGAAGAAAG TGCCAGTTGA TGAGTATATA ACCACGTACC CTGGACAAGG ATGTGCTGGT
      4140       4150       4160       4170       4180       4190       4200

                           >< Tru9I
                           >< MseI
                  >< DdeI     >< Esp4I                        >< RsaI
 >< MnlI               >< BspWI                              >< Csp6I
 >< FokI          >< AluI      >< AflII                 >< Eco57I >< AfaI
TATACACTTG AGGAAGCTAA GACTGCTCTT AAGAAATGCA AATCTGCATT TTATGTACTA CCTTCAGAAG
      4210       4220       4230       4240       4250       4260       4270

                                       >< ScrFI
                                       >< MvaI
                                     >< EcoRII
                    >< XmnI            >< Ecl136I            NlaIII ><
          > < Ksp632I     >< RmaI     >< DsaV               Ksp632I ><
          > < EarI   > < TfiI>< MboII    >< BstOI                    >< EarI
          > < Eam1104I    >< MaeI        >< BstNI            Eam1104I ><
          > < DdeI   > < HinfI           >< BsiLI                 BsmAI ><
      >< BspWI     >< Asp700I           >< ApyI                 Alw26I ><
CACCTAATGC TAAGGAAGAG ATTCTAGGAA CTGTATCCTG GAATTTGAGA GAAATGCTTG CTCATGCTGA
      4280       4290       4300       4310       4320       4330       4340

           >< VspI          >< Zsp2I
           >< Tru9I     >< Ppu10I
           >< MseI          >< NsiI
        >< MboII               >< NlaIII     >< FokI
              >< Eco57I    >< Mph1103I   >< FokI
```

FIGURE 13. 11


>< AsnI
>< AseI
>< EcoT22I
>< AvaIII
>< BspWI
>< BglI
>< MaeII
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4350 4360 4370 4380 4390 4400 4410
>< SfaNI
>< Tru9I
>< MseI
> < HindII
> < HincII
>< MboII
>< TfiI
>< SpeI
>< RmaI
>< MnlI
>< DrdI
>< HinfI
>< MaeI
AAAGGAATTA AAATTCAAGA GGGCATCGTT GACTATGGTG TCCGATTCTT CTTTTATACT AGTAAAGAGC
4420 4430 4440 4450 4460 4470 4480
>< MaeIII
>< SfcI
>< Fnu4HI
>< MunI
>< AluI
>< AluI
>< AciI
MaeIII ><
CTGTAGCTTC TATTATTACG AAGCTGAACT CTCTAAATGA GCCGCTTGTC ACAATGCCAA TTGGTTATGT
4490 4500 4510 4520 4530 4540 4550
>< ThaI
>< MvnI
>< MboII
>< HinP1I
>< HinP1I
>< Hin6I
>< Hin6I
>< HhaI
>< Tru9I
>< HhaI
>< NlaIII
>< Fnu4HI
>< MseI
>< CfoI
>< MnlI
>< CfoI
>< Ksp632I
>< BstUI
>< EarI
>< BssHII
>< BspWI
>< Tru9I
>< Eam1104I
>< Bsp50I
>< MseI
>< BbvI
>< AccII
>< AluI
HphI ><
GACACATGGT TTTAATCTTG AAGAGGCTGC GCGCTGTATG CGTTCTCTTA AAGCTCCTGC CGTAGTGTCA
4560 4570 4580 4590 4600 4610 4620
>< MaeIII
>< SfaNI
>< AlwNI
>< MnlI
>< MnlI
>< DdeI
GTATCATCAC CAGATGCTGT TACTACATAT AATGGATACC TCACTTCGTC ATCAAAGACA TCTGAGGAGC
4630 4640 4650 4660 4670 4680 4690
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< SduI
>< NspII
>< HgiAI
>< Bsp1286I
>< BmyI
>< Alw21I
>< Eco47I
>< Cfr13I
>< Bsi2I
>< Bme18I
>< AvaII
>< AsuI
>< RsaI
>< Csp6I
>< AfaI
ACTTTGTAGA AACAGTTTCT TTGGCTGGCT CTTACAGAGA TTGGTCCTAT TCAGGACAGC GTACAGAGTT
4700 4710 4720 4730 4740 4750 4760
> < TthHB8I
> < TaqI
>< SduI
>< Van91I
>< NspII
>< Tru9I
>< RsaI
>< PflMI
>< Eco24I
>< MseI
>< HphI
>< BslI
>< Bsp1286I
>< Esp4I
>< Csp6I
>< BsiYI
>< BmyI GsuI ><

FIGURE 13.12

```
                ><AflII  ><MaeIII       ><AfaI  ><AccB7I  ><BanIIBpmI ><
AGGTGTTGAA TTTCTTAAGC GTGGTGACAA AATTGTGTAC CACACTCTGG AGAGCCCCGT CGAGTTTCAT
      4770       4780       4790       4800       4810       4820       4830

                                                          ><Tru9I
                                                 ><PleI  ><EcoNI
                                                    ><MnlI ><BslI
                                                 ><BsmAI    ><BsiYI
 ><MnlI          ><HphI              ><HinfI><Alw26I><AciI ><MseI
CTTGACGGTG AGGTTCTTTC ACTTGACAAA CTAAAGAGTC TCTTATCCCT GCGGGAGGTT AAGACTATAA
      4840       4850       4860       4870       4880       4890       4900

                                              ><AluI                ><NdeI
AAGTGTTCAC AACTGTGGAC AACACTAATC TCCACACACA GCTTGTGGAT ATGTCTATGA CATATGGACA
      4910       4920       4930       4940       4950       4960       4970

      ><SinI
      ><Sau96I
      ><NspIV
       ><NspHII
      ><Eco47I
      ><Cfr13I                                                  NlaIII ><
      ><BsiZI                                                 ><NlaIII
      ><Bme18I                                                   > <MnlI
      ><AvaII                        ><MaeIII   ><Tru9I     ><MnlI
      ><AsuI                          ><FokI    ><MseI           ><BspHI
GCAGTTTGGT CCAACATACT TGGATGGTGC TGATGTTACA AAAATTAAAC CTCATGTAAA TCATGAGGGT
      4980       4990       5000       5010       5020       5030       5040

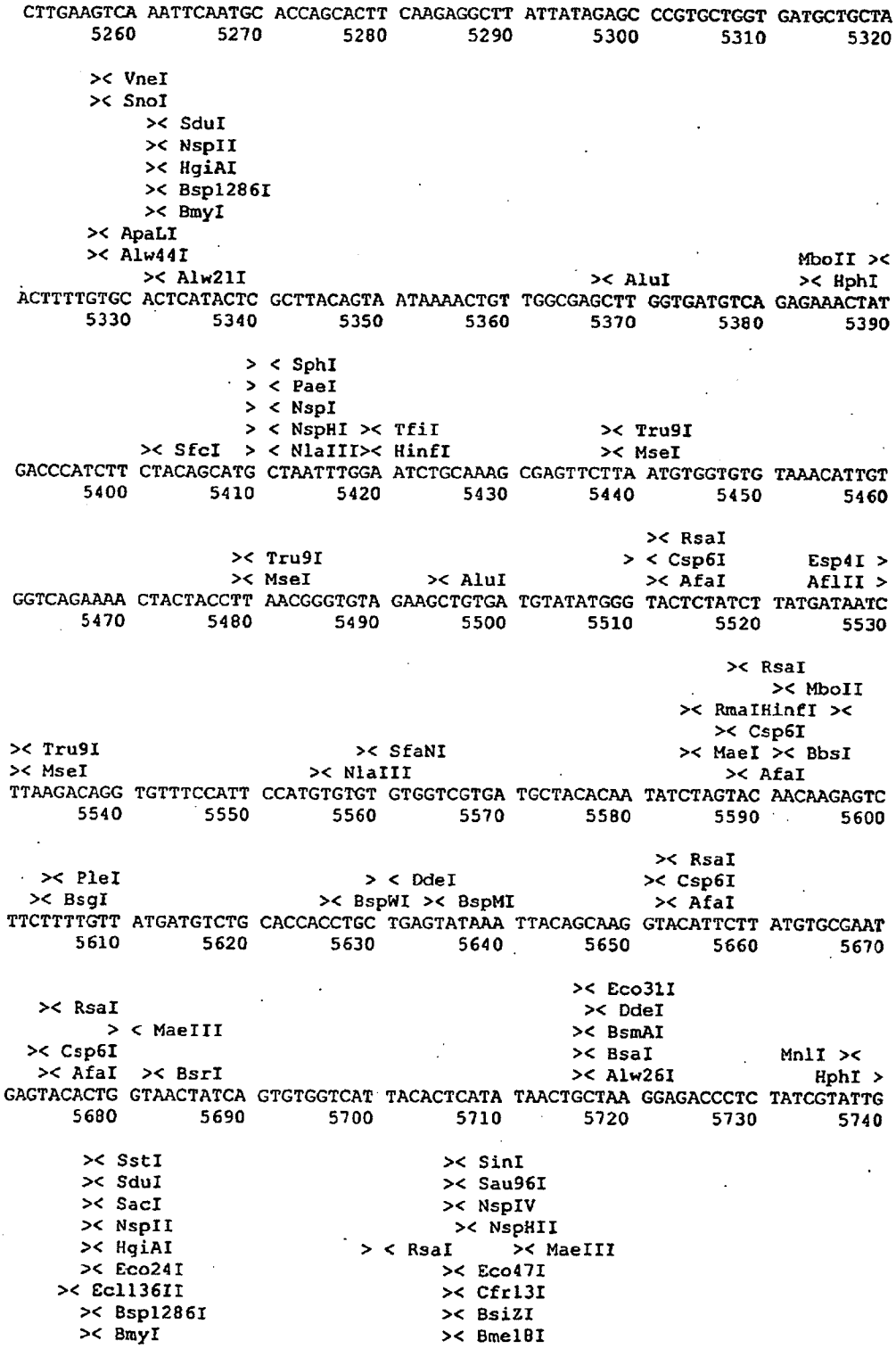
                                                 > <TthHB8I
            ><RsaI                               > <TaqI
                 > <RmaI           ><SnaBI            ><ScaI
                 > <MaeI          ><MaeII ><HindIII  ><RsaI
               ><Csp6I             ><Eco105I         ><Csp6I
                ><AfaI             ><BsaAI  ><AluI    ><AfaI
AAGACTTTCT TTGTACTACC TAGTGATGAC ACACTACGTA GTGAAGCTTT CGAGTACTAC CATACTCTTG
      5050       5060       5070       5080       5090       5100       5110

                  ><RsaI
                       ><NspI
                       ><NspHI
                       ><NlaIII
                  > <Csp6I      ><Tru9I                          MnlI >
                    ><AflIII    ><MseI                         BslI ><
                   ><AfaI        ><DraI                       BsiYI ><
ATGAGAGTTT TCTTGGTAGG TACATGTCTG CTTTAAACCA CACAAAGAAA TGGAAATTTC CTCAAGTTGG
      5120       5130       5140       5150       5160       5170       5180

   ><Tru9I   ><Tru9I                        ><RmaI
   ><MseI    ><MseI          ><MunI         ><MaeI                AluI >
TGGTTTAACT TCAATTAAAT GGGCTGATAA CAATTGTTAT TTGTCTAGTG TTTTATTAGC ACTTCAACAG
      5190       5200       5210       5220       5230       5240       5250

                                                  ><SfaNI
                                                  ><SduI
                                                  ><NspII
                                                  ><Eco24I
                                                  ><Bsp1286I
                                                  ><BmyI          HphI >
                                                  ><BbvI Fnu4HI ><
                          ><MnlI                  ><BanII   ><BspWI
```

FIGURE 13.13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5260 5270 5280 5290 5300 5310 5320
>< VneI
>< SnoI
>< SduI
>< NspII
>< HgiAI
>< Bsp1286I
>< BmyI
>< ApaLI
>< Alw44I
MboII ><
>< Alw21I
>< AluI
>< HphI
ACTTTTGTGC ACTCATACTC GCTTACAGTA ATAAAACTGT TGGCGAGCTT GGTGATGTCA GAGAAACTAT
5330 5340 5350 5360 5370 5380 5390
> < SphI
> < PaeI
> < NspI
> < NspHI >< TfiI
>< Tru9I
>< SfcI > < NlaIII>< HinfI
>< MseI
GACCCATCTT CTACAGCATG CTAATTTGGA ATCTGCAAAG CGAGTTCTTA ATGTGGTGTG TAAACATTGT
5400 5410 5420 5430 5440 5450 5460
>< RsaI
>< Tru9I
> < Csp6I
Esp4I >
>< MseI
>< AluI
>< AfaI
AflII >
GGTCAGAAAA CTACTACCTT AACGGGTGTA GAAGCTGTGA TGTATATGGG TACTCTATCT TATGATAATC
5470 5480 5490 5500 5510 5520 5530
>< RsaI
>< MboII
>< RmaIHinfI ><
>< Csp6I
>< Tru9I
>< SfaNI
>< MaeI >< BbsI
>< MseI
>< NlaIII
>< AfaI
TTAAGACAGG TGTTTCCATT CCATGTGTGT GTGGTCGTGA TGCTACACAA TATCTAGTAC AACAAGAGTC
5540 5550 5560 5570 5580 5590 5600
>< RsaI
>< PleI
> < DdeI
>< Csp6I
>< BsgI
>< BspWI >< BspMI
>< AfaI
TTCTTTTGTT ATGATGTCTG CACCACCTGC TGAGTATAAA TTACAGCAAG GTACATTCTT ATGTGCGAAT
5610 5620 5630 5640 5650 5660 5670
>< Eco31I
>< RsaI
>< DdeI
> < MaeIII
>< BsmAI
>< Csp6I
>< BsaI
MnlI ><
>< AfaI >< BsrI
>< Alw26I
HphI >
GAGTACACTG GTAACTATCA GTGTGGTCAT TACACTCATA TAACTGCTAA GGAGACCCTC TATCGTATTG
5680 5690 5700 5710 5720 5730 5740
>< SstI
>< SinI
>< SduI
>< Sau96I
>< SacI
>< NspIV
>< NspII
>< NspHII
>< HgiAI
> < RsaI >< MaeIII
>< Eco24I
>< Eco47I
>< Ecl136II
>< Cfr13I
>< Bsp1286I
>< BsiZI
>< BmyI
>< BmeI8I

FIGURE 13. 14

```
        >< BanII                          >< AvaII
        >< Alw21I                  >< Csp6I>< AsuI
      >< AluI                       > < AfaI  >< BsrI>< AlwNI
ACGGAGCTCA CCTTACAAAG ATGTCAGAGT ACAAAGGACC AGTGACTGAT GTTTTCTACA AGGAAACATC
      5750        5760        5770        5780        5790        5800        5810

                                        >< TthHB8I
                                        >< TaqI >< MaeIII
TTACACTACA ACCATCAAGC CTGTGTCGTA TAAACTCGAT GGAGTTACTT ACACAGAGAT TGAACCAAAA
      5820        5830        5840        5850        5860        5870        5880

                                                              >< RsaI
                                                             >< Csp6I
                                                    >< SfcI >< BbvI
                  >< FokI                         >< Fnu4HI        >< AfaI
TTGGATGGGT ATTATAAAAA GGATAATGCT TACTATACAG AGCAGCCTAT AGACCTTGTA CCAACTCAAC
      5890        5900        5910        5920        5930        5940        5950

                                                                 Tru9I ><
                                                                   SwaI ><
                                                                  MseI ><
                                          > < NspI                 MamI ><
                                          > < NspHI                DraI ><
                                          > < NlaIII               BsiBI ><
                                       >< AflIII                   BsaBI ><
CATTACCAAA TGCGAGTTTT GATAATTTCA AACTCACATG TTCTAACACA AAATTTGCTG ATGATTTAAA
      5960        5970        5980        5990        6000        6010        6020

                                                 >< MboII
                           >< AluI      >< AluI>< MaeIII
TCAAATGACA GGCTTCACAA AGCCAGCTTC ACGAGAGCTA TCTGTCACAT TCTTCCCAGA CTTGAATGGC
      6030        6040        6050        6060        6070        6080        6090

                    >< SfcI
GATGTAGTGG CTATTGACTA TAGACACTAT TCAGCGAGTT TCAAGAAAGG TGCTAAATTA CTGCATAAGC
      6100        6110        6120        6130        6140        6150        6160

                 >< Tru9I
                      >< ScrFI
                      >< MvaI
                 >< MseI
                    >< EcoRII
                       >< Ecl136I
                    >< DsaV
                       >< BstOI
                       >< BstNI                                    MaeII ><
>< MunI                >< BsiLI                                   >< DraIII
     >< BstXI          >< ApyI              >< MaeII          >< BstXI
CAATTGTTTG GCACATTAAC CAGGCTACAA CCAAGACAAC GTTCAAACCA AACACTTGGT GTTTACGTTG
      6170        6180        6190        6200        6210        6220        6230

        > < RsaI
       >< Csp6I                                                    MboII ><
        > < AfaI>< BsrI                                           >< BbsI
TCTTTGGAGT ACAAAGCCAG TAGATACTTC AAATTCATTT GAAGTTCTGG CAGTAGAAGA CACACAAGGA
      6240        6250        6260        6270        6280        6290        6300

                          >< HindII                        >< MboII
                          >< HincII                  >< MnlI       >< Eco57I
ATGGACAATC TTGCTTGTGA AAGTCAACAA CCCACCTCTG AAGAAGTAGT GGAAAATCCT ACCATACAGA
      6310        6320        6330        6340        6350        6360        6370
```

FIGURE 13.15

```
              >< MaeIII                                          >< Tru9I
                 >< MaeII                                        >< MseI
AGGAAGTCAT AGAGTGTGAC GTGAAAACTA CCGAAGTTGT AGGCAATGTC ATACTTAAAC CATCAGATGA
      6380       6390       6400       6410       6420       6430       6440

                                  >< XhoII
                                  >< Sau3AI
                                ><  NlaIII
                                  >< NdeII
                                  >< MflI
                                  >< MboI
                                  >< DpnII
                                    >< DpnI
                                  >< BstYI
   >< Tru9I                       >< BspAI
   >< MseI                  >< BspHI >< Bsp143I>< Fnu4HI
      > < MaeIII       >< MnlI >< BbvI     >< AlwI
AGGTGTTAAA GTAACACAAG AGTTAGGTCA TGAGGATCTT ATGGCTGCTT ATGTGGAAAA CACAAGCATT
      6450       6460       6470       6480       6490       6500       6510

                                  >< SauI
                                ><  RmaI
                                  >< MstII
                                ><  MaeI
                                  >< Eco81I
                                  >< DdeI
                                  >< CvnI
                                  >< Bsu36I
                                  >< Bse21I
                                  >< BfrI> < Tru9I
   >< Tru9I                       >< AxyI> < MseI>< MunI          >< NlaIII
   >< MseI           >< AluI      >< AocI  >< DraI       >< BbvI Fnu4HI ><
ACCATTAAGA AACCTAATGA GCTTTCACTA GCCTTAGGTT TAAAAACAAT TGCCACTCAT GGTATTGCTG
      6520       6530       6540       6550       6560       6570       6580

  >< VspI     >< StyI
  >< Tru9I    >< EcoT14I                                 > < DdeI
  >< MseI     >< Eco130I                                   >< BslI
  >< AsnI     >< BssT1I                                    >< BsiYI
  >< AseI     >< BsaJI                                   > < BfrI     >< Fnu4HI
CAATTAATAG TGTTCCTTGG AGTAAAATTT TGGCTTATGT CAAACCATTC TTAGGACAAG CAGCAATTAC
      6590       6600       6610       6620       6630       6640       6650

           >< HinP1I
           >< Hin6I                    >< Tru9I
             >< HhaI             >< MaeII>< MseI
              >< DdeI             >< DraIII
 >< BbvI     >< CfoI             >< AflIII
AACATCAAAT TGCGCTAAGA GATTAGCACA ACGTGTGTTT AACAATTATA TGCCTTATGT GTTTACATTA
      6660       6670       6680       6690       6700       6710       6720

             >< RsaI          > < RsaI>< XbaI
            >< Csp6I         >< Csp6I  >< RmaI
     >< MunI  >< AfaI          > < AfaI >< MaeI     >< AluI
TTGTTCCAAT TGTGTACTTT TACTAAAAGT ACCAATTCTA GAATTAGAGC TTCACTACCT ACAACTATTG
      6730       6740       6750       6760       6770       6780       6790

                                                     >< VspI
                                                     >< Tru9I
                                                  >< NaeI
                                                 >< MspI
                                                     >< MseI
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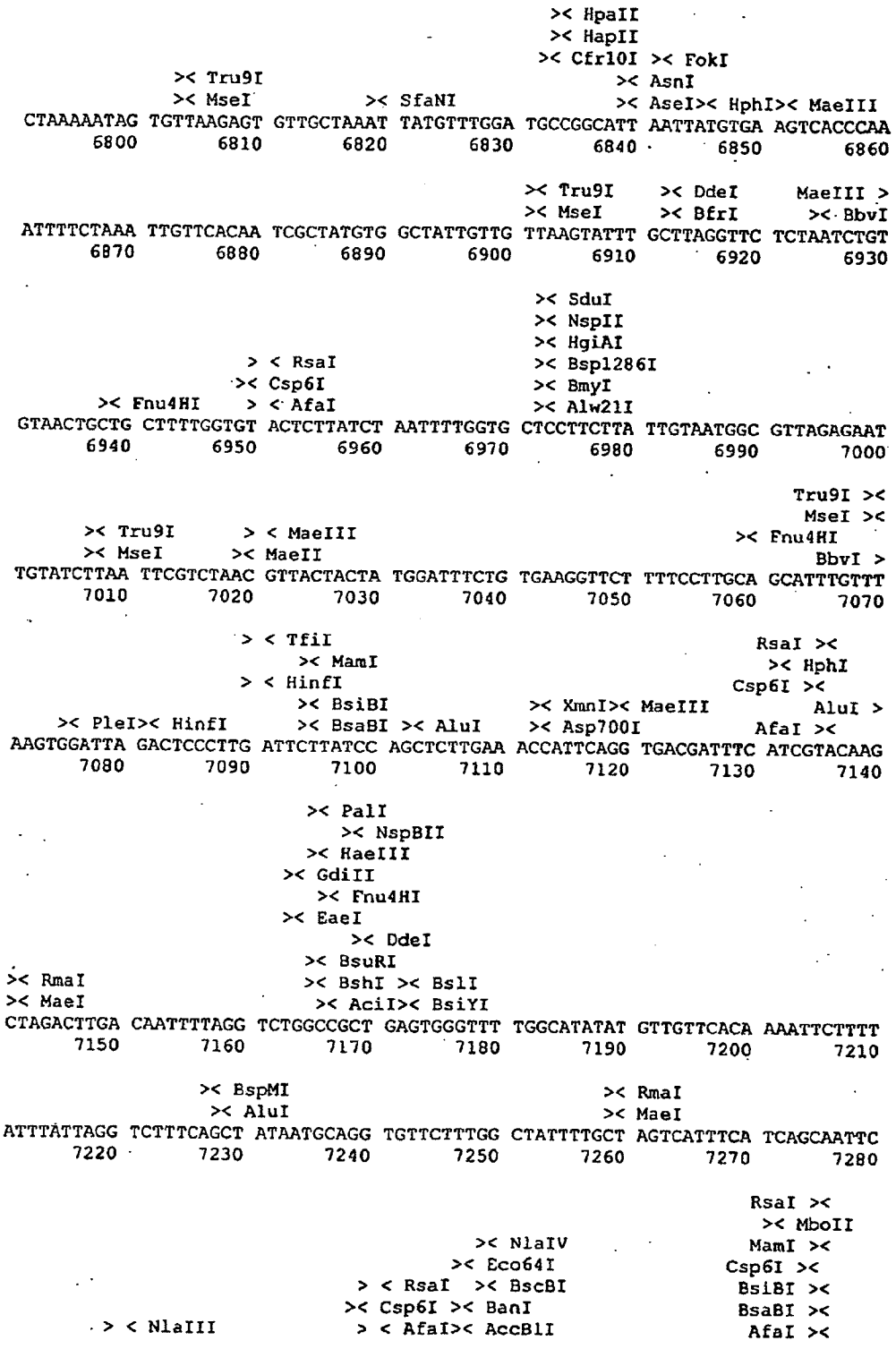
FIGURE 13. 16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>< HapII
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>< Tru9I
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>< AsnI
>< SfaNI
>< AseI>< HphI>< MaeIII
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6800 6810 6820 6830 6840 6850 6860
>< Tru9I >< DdeI MaeIII >
>< MseI >< BfrI >< BbvI
ATTTTCTAAA TTGTTCACAA TCGCTATGTG GCTATTGTTG TTAAGTATTT GCTTAGGTTC TCTAATCTGT
6870 6880 6890 6900 6910 6920 6930
>< SduI
>< NspII
>< HgiAI
> < RsaI >< Bspl286I
>< Csp6I >< BmyI
>< Fnu4HI > < AfaI >< Alw21I
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6940 6950 6960 6970 6980 6990 7000
Tru9I ><
MseI ><
>< Tru9I > < MaeIII >< Fnu4HI
>< MseI >< MaeII BbvI >
TGTATCTTAA TTCGTCTAAC GTTACTACTA TGGATTTCTG TGAAGGTTCT TTTCCTTGCA GCATTTGTTT
7010 7020 7030 7040 7050 7060 7070
> < TfiI RsaI ><
>< MamI >< HphI
> < HinfI Csp6I ><
>< BsiBI >< XmnI>< MaeIII AluI >
>< PleI>< HinfI >< BsaBI >< AluI >< Asp700I AfaI ><
AAGTGGATTA GACTCCCTTG ATTCTTATCC AGCTCTTGAA ACCATTCAGG TGACGATTTC ATCGTACAAG
7080 7090 7100 7110 7120 7130 7140
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>< NspBII
>< HaeIII
>< GdiII
>< Fnu4HI
>< EaeI
>< DdeI
>< BsuRI
>< RmaI >< BshI >< BslI
>< MaeI >< AciI>< BsiYI
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7150 7160 7170 7180 7190 7200 7210
>< BspMI >< RmaI
>< AluI >< MaeI
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7220 7230 7240 7250 7260 7270 7280
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>< MboII
>< NlaIV MamI ><
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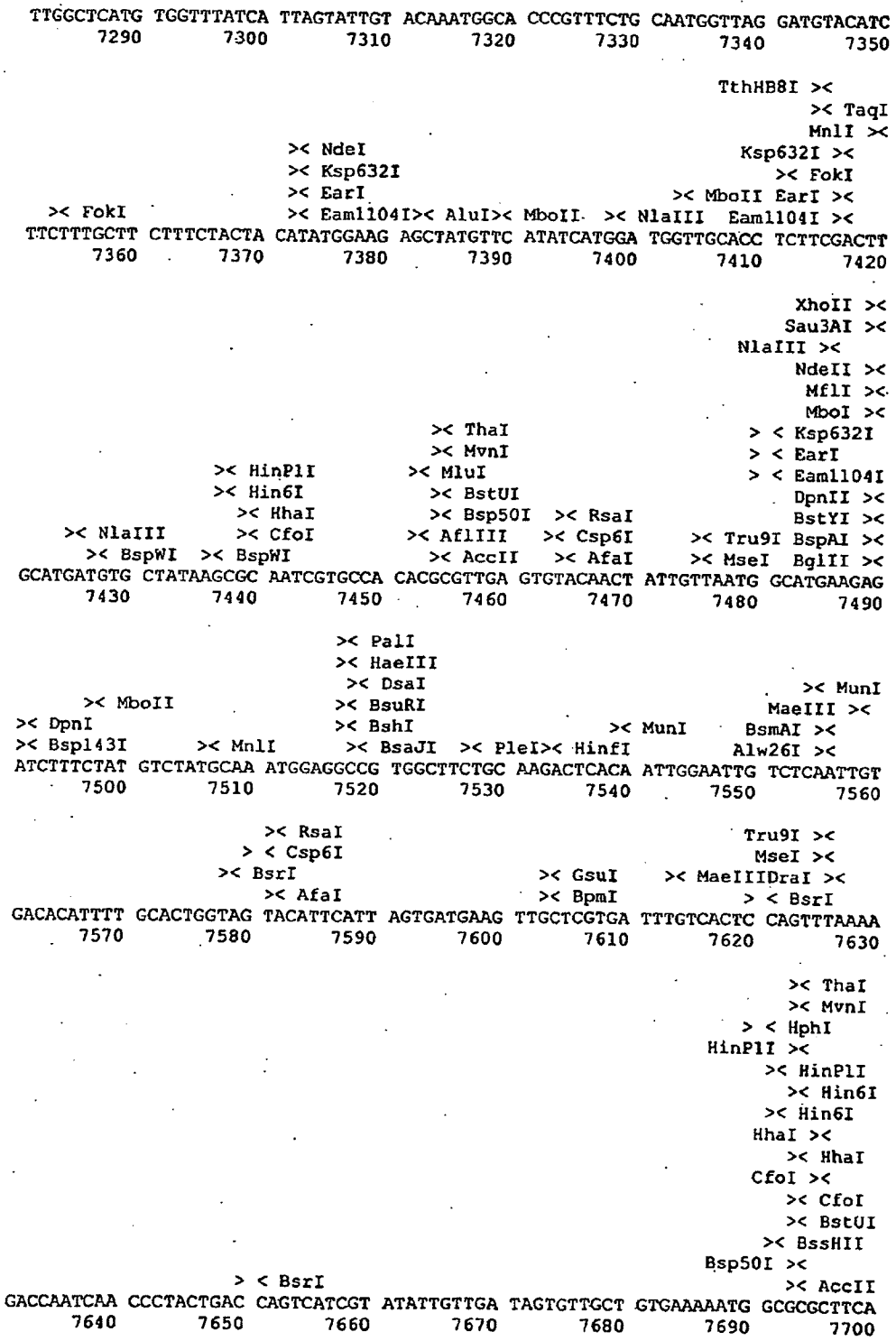
> < NlaIII > < AfaI>< AccBlI AfaI ><

FIGURE 13.17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>< TaqI
MnlI ><
>< NdeI
>< Ksp632I
>< EarI
Ksp632I ><
>< FokI
>< MboII EarI ><
>< FokI
>< Eam1104I>< AluI>< MboII >< NlaIII Eam1104I ><
TTCTTTGCTT CTTTCTACTA CATATGGAAG AGCTATGTTC ATATCATGGA TGGTTGCACC TCTTCGACTT
7360 7370 7380 7390 7400 7410 7420
XhoII ><
Sau3AI ><
NlaIII ><
NdeII ><
MflI ><
MboI ><
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>< MvnI
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>< Hin6I
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DpnII ><
>< HhaI
>< Bsp50I >< RsaI
BstYI ><
>< NlaIII
>< CfoI
>< AflIII >< Csp6I
>< Tru9I BspAI ><
>< BspWI >< BspWI
>< AccII >< AfaI
>< MseI BglII ><
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7430 7440 7450 7460 7470 7480 7490
>< PalI
>< HaeIII
>< DsaI
>< MunI
>< MboII
>< BsuRI
MaeIII ><
>< DpnI
>< BshI
>< MunI
BsmAI ><
>< Bsp143I
>< MnlI
>< BsaJI >< PleI>< HinfI
Alw26I ><
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7500 7510 7520 7530 7540 7550 7560
>< RsaI
Tru9I ><
> < Csp6I
MseI ><
>< BsrI
>< GsuI
>< MaeIIIDraI ><
>< AfaI
>< BpmI
> < BsrI
GACACATTTT GCACTGGTAG TACATTCATT AGTGATGAAG TTGCTCGTGA TTTGTCACTC CAGTTTAAAA
7570 7580 7590 7600 7610 7620 7630
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HhaI ><
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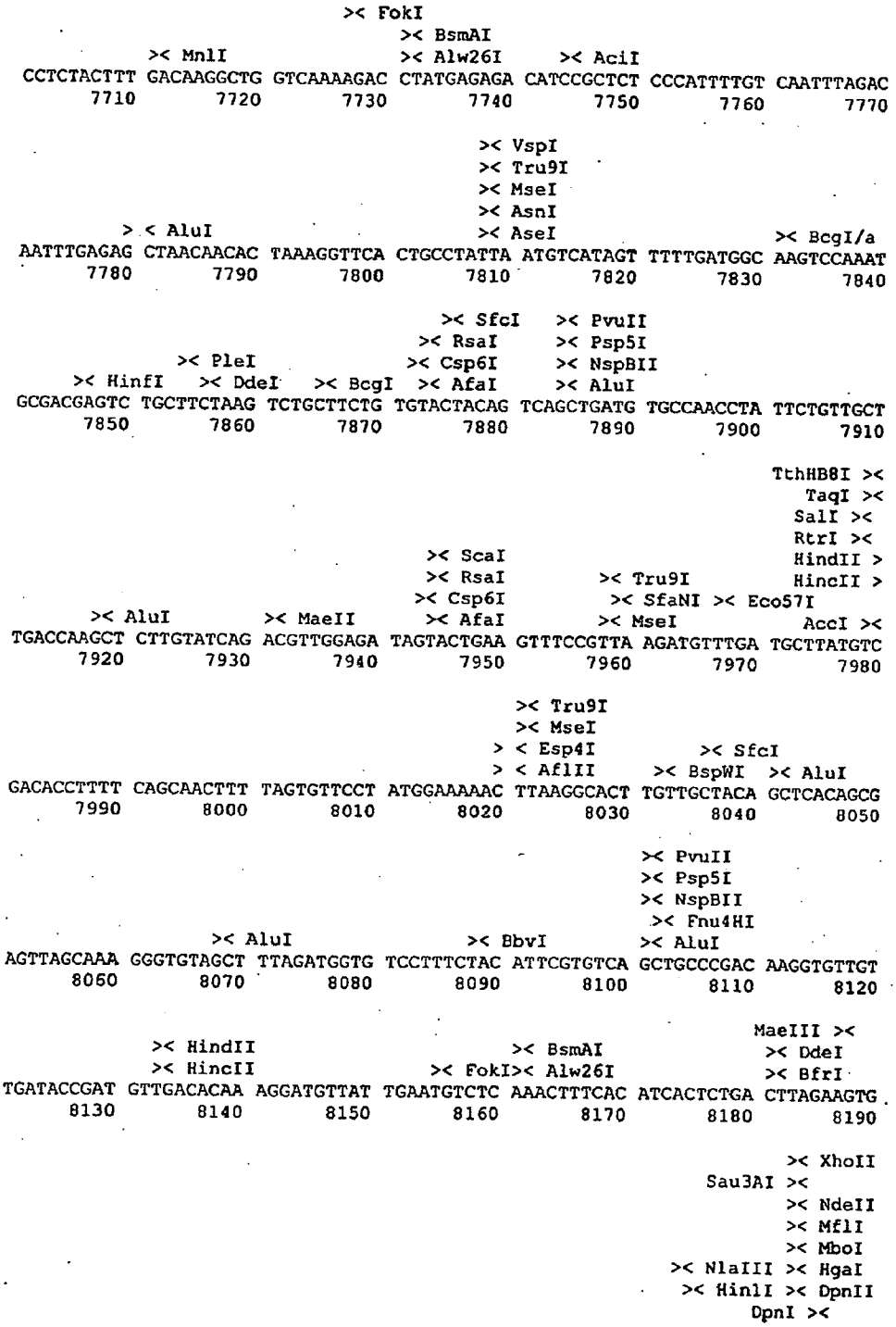
7640 7650 7660 7670 7680 7690 7700

FIGURE 13. 18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7710 7720 7730 7740 7750 7760 7770
>< VspI
>< Tru9I
>< MseI
>< AsnI
> < AluI
>< AseI
>< BcgI/a
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7780 7790 7800 7810 7820 7830 7840
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>< PvuII
>< RsaI
>< Psp5I
>< PleI
>< Csp6I
>< NspBII
>< HinfI
>< DdeI
>< BcgI
>< AfaI
>< AluI
GCGACGAGTC TGCTTCTAAG TCTGCTTCTG TGTACTACAG TCAGCTGATG TGCCAACCTA TTCTGTTGCT
7850 7860 7870 7880 7890 7900 7910
TthHB8I ><
TaqI ><
SalI ><
RtrI ><
>< ScaI
HindII >
>< RsaI
>< Tru9I
HincII >
>< Csp6I
>< SfaNI >< Eco57I
>< AluI
>< MaeII
>< AfaI
>< MseI
AccI ><
TGACCAAGCT CTTGTATCAG ACGTTGGAGA TAGTACTGAA GTTTCCGTTA AGATGTTTGA TGCTTATGTC
7920 7930 7940 7950 7960 7970 7980
>< Tru9I
>< MseI
> < Esp4I
>< SfcI
> < AflII
>< BspWI >< AluI
GACACCTTTT CAGCAACTTT TAGTGTTCCT ATGGAAAAAC TTAAGGCACT TGTTGCTACA GCTCACAGCG
7990 8000 8010 8020 8030 8040 8050
>< PvuII
>< Psp5I
>< NspBII
>< Fnu4HI
>< AluI
>< AluI
>< BbvI
>< AluI
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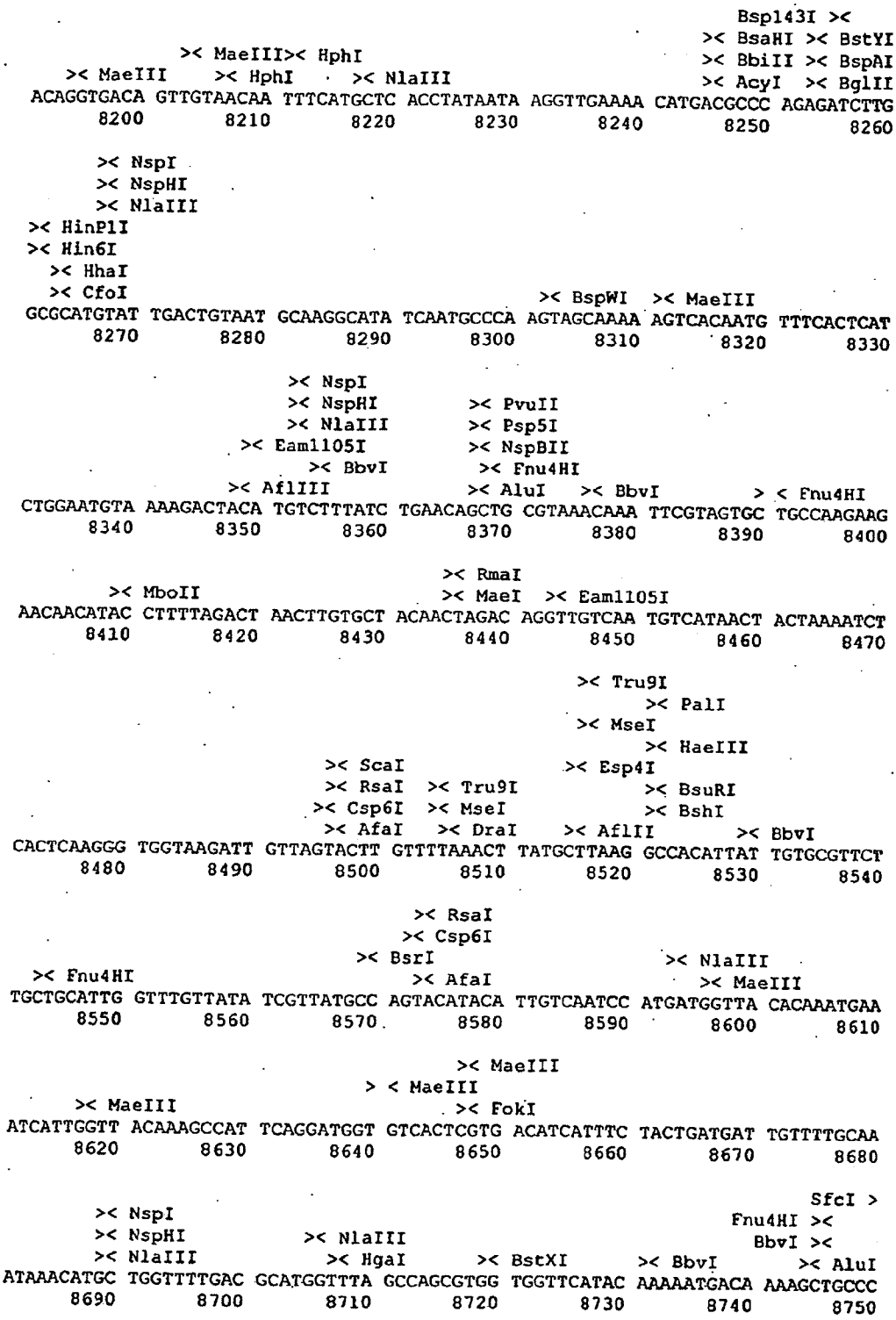
8060 8070 8080 8090 8100 8110 8120
MaeIII ><
>< HindII
>< DdeI
>< HincII
>< BsmAI
>< BfrI
>< FokI>< Alw26I
TGATACCGAT GTTGACACAA AGGATGTTAT TGAATGTCTC AAACTTTCAC ATCACTCTGA CTTAGAAGTG
8130 8140 8150 8160 8170 8180 8190
>< XhoII
Sau3AI ><
>< NdeII
>< MflI
>< MboI
>< NlaIII >< HgaI
>< HinlI >< DpnII
DpnI ><

FIGURE 13.19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8200 8210 8220 8230 8240 8250 8260
>< NspI
>< NspHI
>< NlaIII
>< HinP1I
>< Hin6I
>< HhaI
>< CfoI
>< BspWI >< MaeIII
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8270 8280 8290 8300 8310 8320 8330
>< NspI
>< NspHI >< PvuII
>< NlaIII >< Psp5I
>< Eam1105I >< NspBII
>< BbvI >< Fnu4HI
>< AflIII >< AluI >< BbvI > < Fnu4HI
CTGGAATGTA AAAGACTACA TGTCTTTATC TGAACAGCTG CGTAAACAAA TTCGTAGTGC TGCCAAGAAG
8340 8350 8360 8370 8380 8390 8400
>< RmaI
>< MboII >< MaeI >< Eam1105I
AACAACATAC CTTTTAGACT AACTTGTGCT ACAACTAGAC AGGTTGTCAA TGTCATAACT ACTAAAATCT
8410 8420 8430 8440 8450 8460 8470
>< Tru9I
>< PalI
>< MseI
>< HaeIII
>< ScaI >< Esp4I
>< RsaI >< Tru9I >< BsuRI
>< Csp6I >< MseI >< BshI
>< AfaI >< DraI >< AflII >< BbvI
CACTCAAGGG TGGTAAGATT GTTAGTACTT GTTTTAAACT TATGCTTAAG GCCACATTAT TGTGCGTTCT
8480 8490 8500 8510 8520 8530 8540
>< RsaI
>< Csp6I
>< BsrI >< NlaIII
>< Fnu4HI >< AfaI >< MaeIII
TGCTGCATTG GTTTGTTATA TCGTTATGCC AGTACATACA TTGTCAATCC ATGATGGTTA CACAAATGAA
8550 8560 8570 8580 8590 8600 8610
>< MaeIII
> < MaeIII
>< MaeIII >< FokI
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8620 8630 8640 8650 8660 8670 8680
SfcI >
>< NspI Fnu4HI ><
>< NspHI >< NlaIII BbvI ><
>< NlaIII >< HgaI >< BstXI >< BbvI >< AluI
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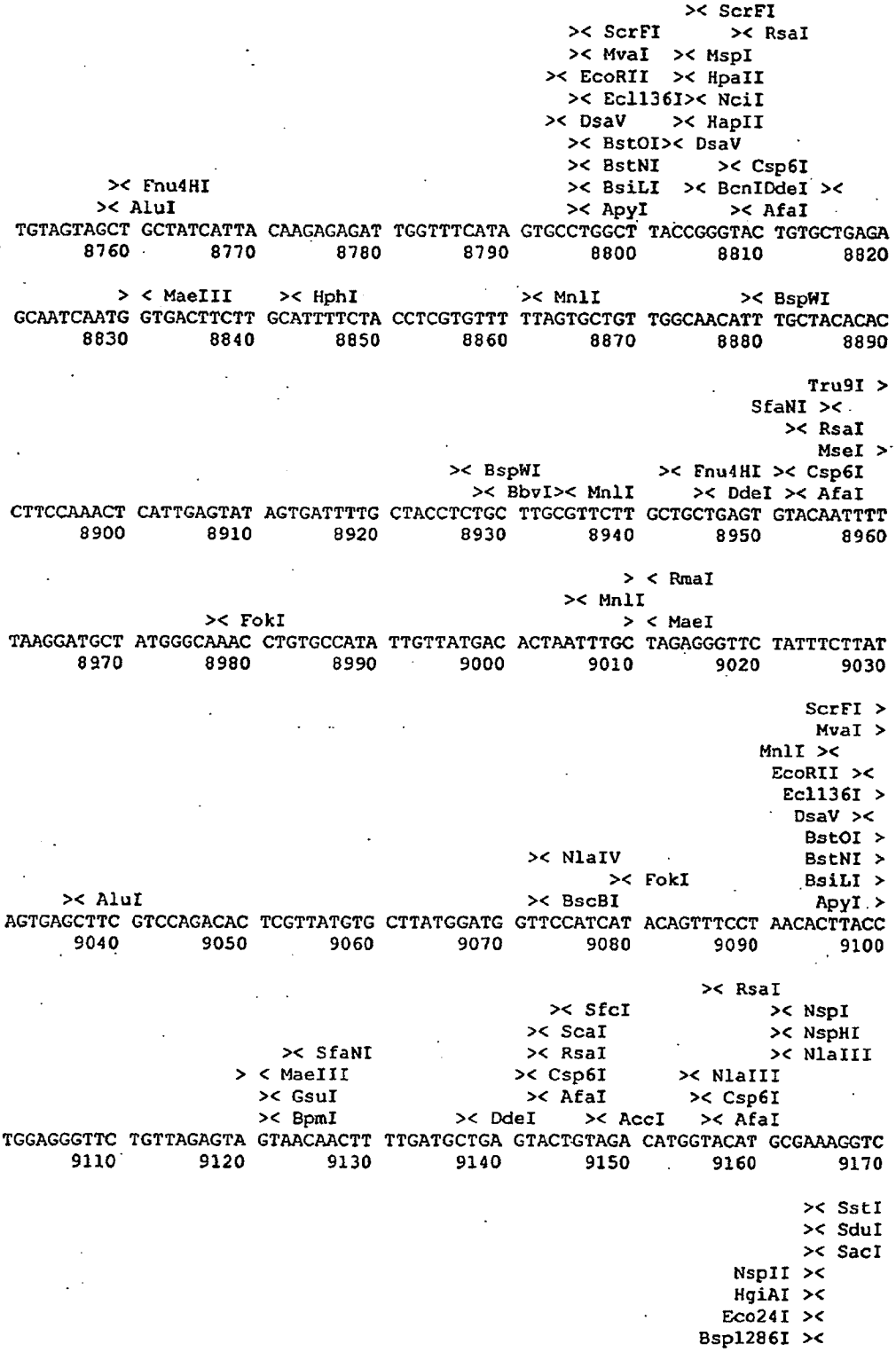
8690 8700 8710 8720 8730 8740 8750

FIGURE 13. 20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>< Fnu4HI >< BsiLI >< BcnIDdeI ><
>< AluI >< ApyI >< AfaI
TGTAGTAGCT GCTATCATTA CAAGAGAGAT TGGTTTCATA GTGCCTGGCT TACCGGGTAC TGTGCTGAGA
8760 8770 8780 8790 8800 8810 8820
> < MaeIII >< HphI >< MnlI >< BspWI
GCAATCAATG GTGACTTCTT GCATTTTCTA CCTCGTGTTT TTAGTGCTGT TGGCAACATT TGCTACACAC
8830 8840 8850 8860 8870 8880 8890
Tru9I >
SfaNI ><
>< RsaI
MseI >
>< BspWI >< Fnu4HI >< Csp6I
>< BbvI>< MnlI >< DdeI >< AfaI
CTTCCAAACT CATTGAGTAT AGTGATTTTG CTACCTCTGC TTGCGTTCTT GCTGCTGAGT GTACAATTTT
8900 8910 8920 8930 8940 8950 8960
> < RmaI
>< MnlI
>< FokI > < MaeI
TAAGGATGCT ATGGGCAAAC CTGTGCCATA TTGTTATGAC ACTAATTTGC TAGAGGGTTC TATTTCTTAT
8970 8980 8990 9000 9010 9020 9030
ScrFI >
MvaI >
MnlI ><
EcoRII ><
Ecl136I >
DsaV ><
BstOI >
>< NlaIV BstNI >
>< FokI BsiLI >
>< AluI >< BscBI ApyI >
AGTGAGCTTC GTCCAGACAC TCGTTATGTG CTTATGGATG GTTCCATCAT ACAGTTTCCT AACACTTACC
9040 9050 9060 9070 9080 9090 9100
>< RsaI
>< SfcI >< NspI
>< ScaI >< NspHI
>< SfaNI >< RsaI >< NlaIII
> < MaeIII >< Csp6I >< NlaIII
>< GsuI >< AfaI >< Csp6I
>< BpmI >< DdeI >< AccI >< AfaI
TGGAGGGTTC TGTTAGAGTA GTAACAACTT TTGATGCTGA GTACTGTAGA CATGGTACAT GCGAAAGGTC
9110 9120 9130 9140 9150 9160 9170
>< SstI
>< SduI
>< SacI
NspII ><
HgiAI ><
Eco24I ><
Bsp1286I ><

FIGURE 13.21

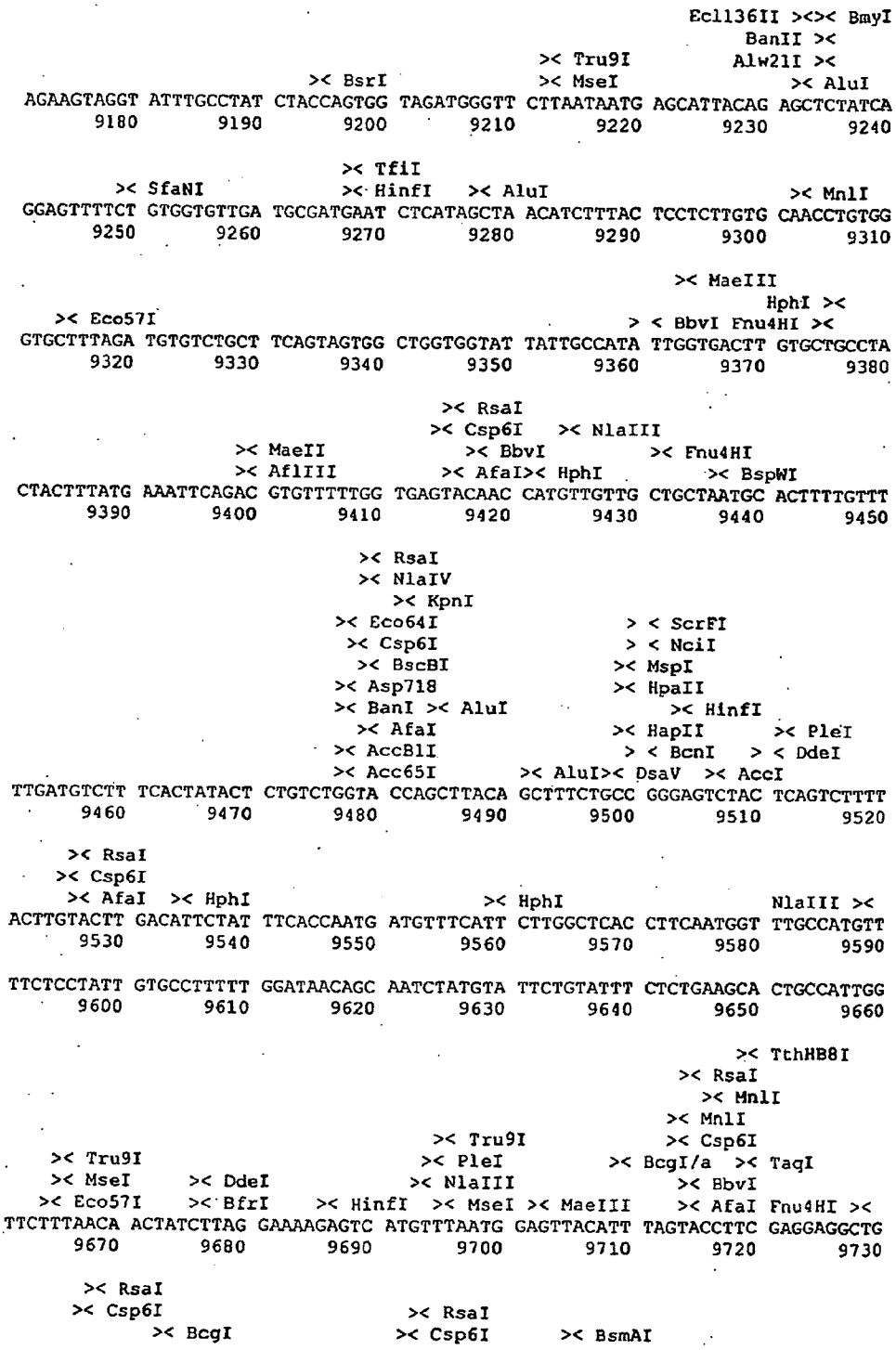
Ecl136II >< >< BmyI
BanII ><
>< Tru9I Alw21I ><
>< BsrI >< MseI >< AluI
AGAAGTAGGT ATTTGCCTAT CTACCAGTGG TAGATGGGTT CTTAATAATG AGCATTACAG AGCTCTATCA
9180 9190 9200 9210 9220 9230 9240
>< TfiI
>< SfaNI >< HinfI >< AluI >< MnlI
GGAGTTTTCT GTGGTGTTGA TGCGATGAAT CTCATAGCTA ACATCTTTAC TCCTCTTGTG CAACCTGTGG
9250 9260 9270 9280 9290 9300 9310
>< MaeIII
HphI ><
>< Eco57I > < BbvI Fnu4HI ><
GTGCTTTAGA TGTGTCTGCT TCAGTAGTGG CTGGTGGTAT TATTGCCATA TTGGTGACTT GTGCTGCCTA
9320 9330 9340 9350 9360 9370 9380
>< RsaI
>< Csp6I >< NlaIII
>< MaeII >< BbvI >< Fnu4HI
>< AflIII >< AfaI >< HphI >< BspWI
CTACTTTATG AAATTCAGAC GTGTTTTTGG TGAGTACAAC CATGTTGTTG CTGCTAATGC ACTTTTGTTT
9390 9400 9410 9420 9430 9440 9450
>< RsaI
>< NlaIV
>< KpnI
>< Eco64I > < ScrFI
>< Csp6I > < NciI
>< BscBI >< MspI
>< Asp718 >< HpaII
>< BanI >< AluI >< HinfI
>< AfaI >< HapII >< PleI
>< Acc65I > < BcnI > < DdeI
>< AluI >< DsaV >< AccI
TTGATGTCTT TCACTATACT CTGTCTGGTA CCAGCTTACA GCTTTCTGCC GGGAGTCTAC TCAGTCTTTT
9460 9470 9480 9490 9500 9510 9520
>< RsaI
>< Csp6I
>< AfaI >< HphI >< HphI NlaIII ><
ACTTGTACTT GACATTCTAT TTCACCAATG ATGTTTCATT CTTGGCTCAC CTTCAATGGT TTGCCATGTT
9530 9540 9550 9560 9570 9580 9590
TTCTCCTATT GTGCCTTTTT GGATAACAGC AATCTATGTA TTCTGTATTT CTCTGAAGCA CTGCCATTGG
9600 9610 9620 9630 9640 9650 9660
>< TthHB8I
>< RsaI
>< MnlI
>< MnlI
>< Tru9I >< Csp6I
>< Tru9I >< PleI >< BcgI/a >< TaqI
>< MseI >< DdeI >< NlaIII >< BbvI
>< Eco57I >< BfrI >< HinfI >< MseI >< MaeIII >< AfaI Fnu4HI ><
TTCTTTAACA ACTATCTTAG GAAAAGAGTC ATGTTTAATG GAGTTACATT TAGTACCTTC GAGGAGGCTG
9670 9680 9690 9700 9710 9720 9730
>< RsaI
>< Csp6I
>< RsaI
>< BcgI >< Csp6I >< BsmAI

FIGURE 13.22

```
       >< AfaI                        >< AfaI      >< Alw26I
CTTTGTGTAC CTTTTTGCTC AACAAGGAAA TGTACCTAAA ATTGCGTAGC GAGACACTGT TGCCACTTAC
      9740       9750       9760       9770       9780       9790       9800

                                                    >< NlaIV
                                    >< RsaI            >< DdeI
                                   >< Csp6I          >< BscBI
                                    >< AfaI             >< BfrI   AluI ><
ACAGTATAAC AGGTATCTTG CTCTATATAA CAAGTACAAG TATTTCAGTG GAGCCTTAGA TACTACCAGC
      9810       9820       9830       9840       9850       9860       9870

                 >< Fnu4HI
                       >< DdeI
             >< Fnu4HI     >< BfrI
   >< BbvI    >< AluI    >< BbvI                              >< DdeI >< AlwNI
TATCGTGAAG CAGCTTGCTG CCACTTAGCA AAGGCTCTAA ATGACTTTAG CAACTCAGGT GCTGATGTTC
      9880       9890       9900       9910       9920       9930       9940

                                        >< SfcI                      >< BsmI
                                             >< PstI          >< BscCI
TCTACCAACC ACCACAGACA TCAATCACTT CTGCTGTTCT GCAGAGTGGT TTTAGGAAAA TGGCATTCCC
      9950       9960       9970       9980       9990      10000      10010

                          >< RsaI
                         >< NlaIII
                               >< MaeIII
                         >< Csp6I                      >< Tru9I
                          >< AfaI                      >< MseI
GTCAGGCAAA GTTGAAGGGT GCATGGTACA AGTAACCTGT GGAACTACAA CTCTTAATGG ATTGTGGTTG
     10020      10030      10040      10050      10060      10070      10080

                                                                 XhoII ><
                                                                 Sau3AI ><
                                                    >< Tru9I     NdeII ><
                                                  >< NspI        MflI ><
                                                  >< NspHI       MboI ><
                          >< NspI                 >< NlaIII      DpnII ><
            >< FokI       >< NspHI                  >< MseI      BstYI ><
           >< Bst1107I    >< NlaIII                   >< MboII   BspAI ><
          >< AccI       >< AflIII                 > < BbsI      BglII ><
GATGACACAG TATACTGTCC AAGACATGTC ATTTGCACAG CAGAAGACAT GCTTAATCCT AACTATGAAG
     10090      10100      10110      10120      10130      10140      10150

                                                                  PalI >
                                                                  MscI >
                                                                 HaeIII >
                                                                 EaeI ><
                                                                  BsuRI >
><  DpnI >< MboII                                                 BshI >
><  Bsp143I                  >< AluI                              BalI >
ATCTGCTCAT TCGCAAATCC AACCATAGCT TTCTTGTTCA GGCTGGCAAT GTTCAACTTC GTGTTATTGG
     10160      10170      10180      10190      10200      10210      10220

                       >< DdeI> < Tru9I
                       >< BfrI> < MseI                 >< DdeI
CCATTCTATG CAAAATTGTC TGCTTAGGCT TAAAGTTGAT ACTTCTAACC CTAAGACACC CAAGTATAAA
     10230      10240      10250      10260      10270      10280      10290

                  >< ScrFI
                  >< MvaI
                 >< EcoRII
                   >< Ecl136I                    >< SphI
```

FIGURE 13.23

```
                  >< DsaV                                  >< PaeI
                    >< BstOI                               >< NspI
                    >< BstNI                               >< NspHI
                    >< BsiLI                        >< RmaI >< NlaIII
                    >< ApyI                         >< MaeI    >< HphI
TTTGTCCGTA TCCAACCTGG TCAAACATTT TCAGTTCTAG CATGCTACAA TGGTTCACCA TCTGGTGTTT
     10300      10310      10320      10330      10340      10350      10360

                                                          >< Sau3AI
                                                          >< NdeII
                                                          >< MboI>< NlaIII
       >< Eco31I                                          >< DpnII
       >< BsmAI                                        >< Tru9I>< DpnI
       >< BsaI>< NlaIII                  >< Tru9I      >< MseI >< Bsp143I
       >< Alw26I                         >< MseI                >< BspAI>< AlwI
ATCAGTGTGC CATGAGACCT AATCATACCA TTAAAGGTTC TTTCCTTAAT GGATCATGTG GTAGTGTTGG
     10370      10380      10390      10400      10410      10420      10430

                                              >< Zsp2I
                                           >< Ppu10I
                                              >< NsiI>< SfaNI
                                                 >< NdeI
                                              >< Mph1103I          RsaI ><
  >< Tru9I                                    >< EcoT22I         Csp6I ><
  >< MseI                                  > < AvaIII    >< AluI   AfaI ><
TTTTAACATT GATTATGATT GCGTGTCTTT CTGCTATATG CATCATATGG AGCTTCCAAC AGGAGTACAC
     10440      10450      10460      10470      10480      10490      10500

                                  >< SinI
                                  >< Sau96I
                                  >< NspIV
                                   >< NspHII                          >< SfcI
                                  >< Eco47I                             RsaI ><
                                  >< Cfr13I                           PstI ><
                                  >< BsiZI                             >< Fnu4HI
    >< RsaI                       >< Bme18I    >< HindII               Csp6I ><
   >< Csp6I>< DdeI                >< AvaII     >< HincII             >< BspWI
    >< AfaI>< BfrI                >< AsuI>< BsgI     >< BbvI >< BspMI  AfaI ><
GCTGGTACTG ACTTAGAAGG TAAATTCTAT GGTCCATTTG TTGACAGACA AACTGCACAG GCTGCAGGTA
     10510      10520      10530      10540      10550      10560      10570

                  >< Tru9I          >< NlaIII
                  >< MseI     >< BbvI         >< Fnu4HI                HphI ><
CAGACACAAC CATAACATTA AATGTTTTGG CATGGCTGTA TGCTGCTGTT ATCAATGGTG ATAGGTGGTT
     10580      10590      10600      10610      10620      10630      10640

  >< Tru9I
       >< TfiI
  >< MseI                                                  >< RsaI
   >< HphI                          >< Tru9I               >< Csp6I
        >< HinfI                    >< MseI                 >< AfaI
TCTTAATAGA TTCACCACTA CTTTGAATGA CTTTAACCTT GTGGCAATGA AGTACAACTA TGAACCTTTG
     10650      10660      10670      10680      10690      10700      10710

                        >< SinI
                        >< Sau96I
                            >< PssI
                         >< Psp5II
                        >< PpuMI
                        >< NspIV
                         >< NspHII
                         >< NlaIV
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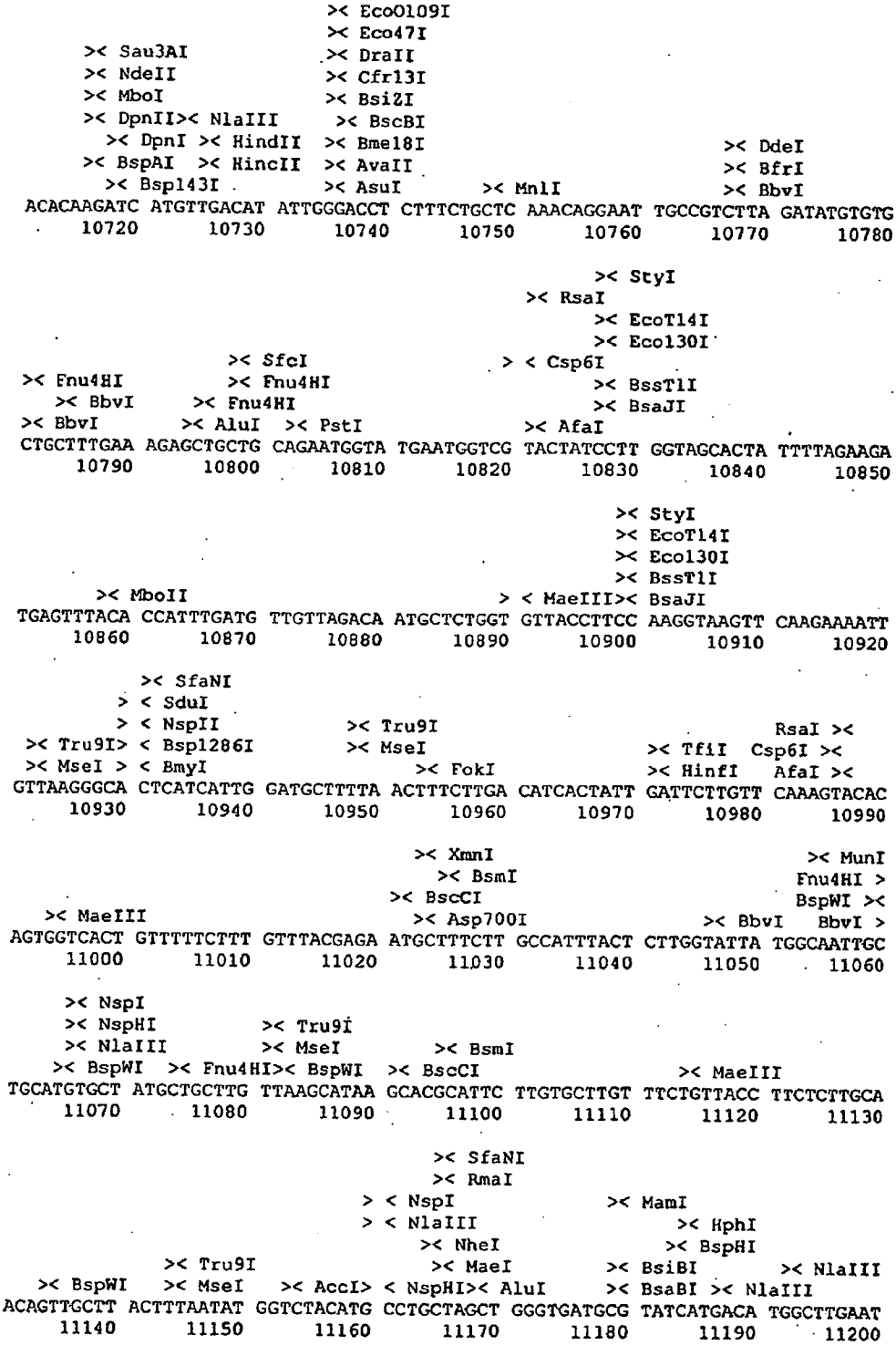
FIGURE 13. 24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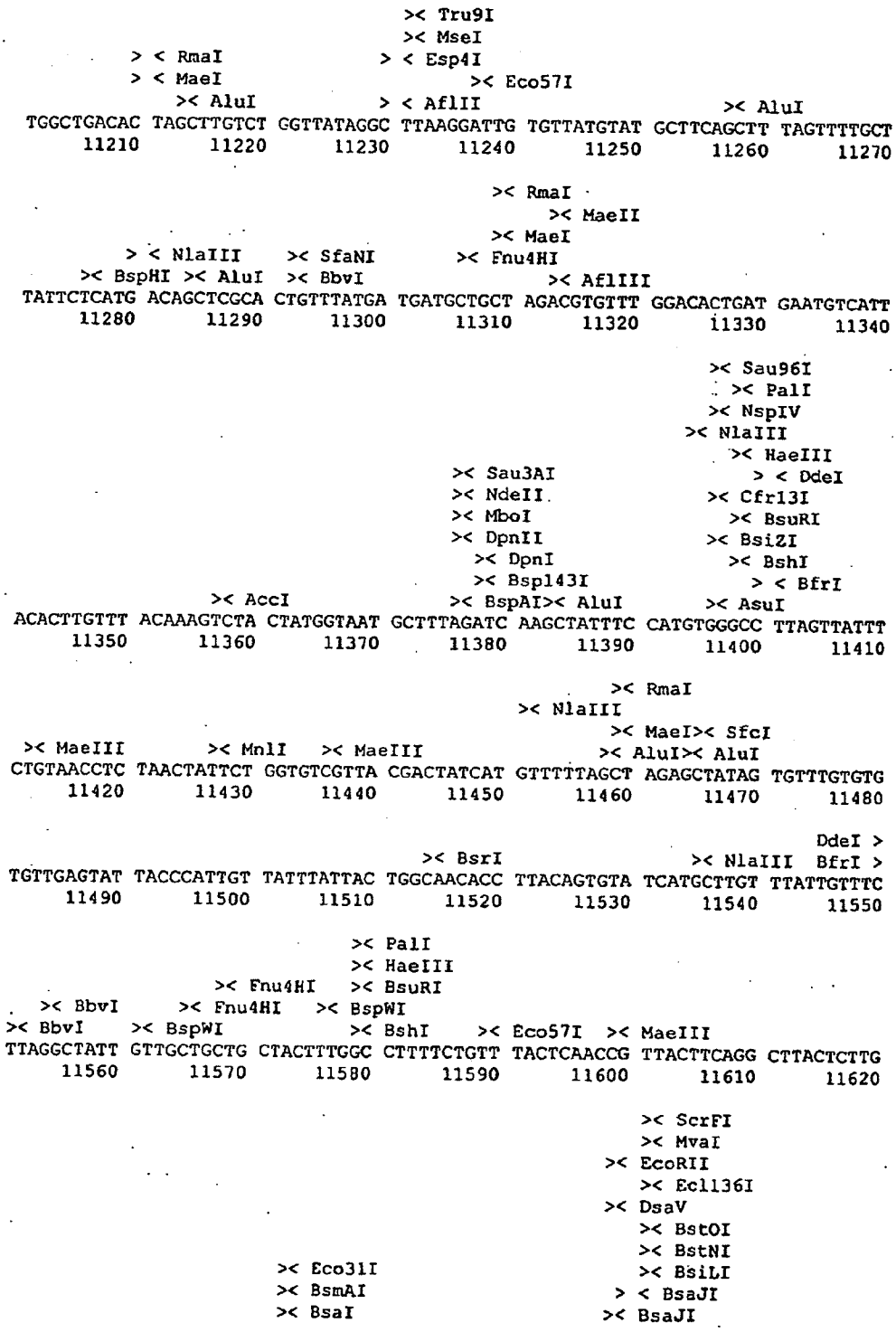
>< Eco47I
>< Sau3AI
>< DraII
>< NdeII
>< Cfr13I
>< MboI
>< Bsi2I
>< DpnII>< NlaIII
>< BscBI
>< DpnI >< HindII
>< Bme18I
>< DdeI
>< BspAI >< HincII
>< AvaII
>< BfrI
>< Bsp143I
>< AsuI
>< MnlI
>< BbvI
ACACAAGATC ATGTTGACAT ATTGGGACCT CTTTCTGCTC AAACAGGAAT TGCCGTCTTA GATATGTGTG
10720 10730 10740 10750 10760 10770 10780
>< StyI
>< RsaI
>< EcoT14I
>< Eco130I
>< SfcI
> < Csp6I
>< Fnu4HI
>< Fnu4HI
>< BssT1I
>< BbvI
>< Fnu4HI
>< BsaJI
>< BbvI
>< AluI
>< PstI
>< AfaI
CTGCTTTGAA AGAGCTGCTG CAGAATGGTA TGAATGGTCG TACTATCCTT GGTAGCACTA TTTTAGAAGA
10790 10800 10810 10820 10830 10840 10850
>< StyI
>< EcoT14I
>< Eco130I
>< BssT1I
>< MboII
> < MaeIII>< BsaJI
TGAGTTTACA CCATTTGATG TTGTTAGACA ATGCTCTGGT GTTACCTTCC AAGGTAAGTT CAAGAAAATT
10860 10870 10880 10890 10900 10910 10920
>< SfaNI
> < SduI
> < NspII
>< Tru9I
RsaI ><
>< Tru9I> < Bsp1286I
>< MseI
>< TfiI Csp6I ><
>< MseI > < BmyI
>< FokI
>< HinfI AfaI ><
GTTAAGGGCA CTCATCATTG GATGCTTTTA ACTTTCTTGA CATCACTATT GATTCTTGTT CAAAGTACAC
10930 10940 10950 10960 10970 10980 10990
>< XmnI
>< MunI
>< BsmI
Fnu4HI >
>< BscCI
BspWI ><
>< MaeIII
>< Asp700I
>< BbvI BbvI >
AGTGGTCACT GTTTTTCTTT GTTTACGAGA ATGCTTTCTT GCCATTTACT CTTGGTATTA TGGCAATTGC
11000 11010 11020 11030 11040 11050 11060
>< NspI
>< NspHI
>< Tru9I
>< NlaIII
>< MseI
>< BsmI
>< BspWI >< Fnu4HI>< BspWI >< BscCI
>< MaeIII
TGCATGTGCT ATGCTGCTTG TTAAGCATAA GCACGCATTC TTGTGCTTGT TTCTGTTACC TTCTCTTGCA
11070 11080 11090 11100 11110 11120 11130
>< SfaNI
>< RmaI
> < NspI
>< MamI
> < NlaIII
>< HphI
>< NheI
>< BspHI
>< Tru9I
>< MaeI
>< BsiBI
>< NlaIII
>< BspWI >< MseI >< AccI> < NspHI>< AluI
>< BsaBI >< NlaIII
ACAGTTGCTT ACTTTAATAT GGTCTACATG CCTGCTAGCT GGGTGATGCG TATCATGACA TGGCTTGAAT
11140 11150 11160 11170 11180 11190 11200

FIGURE 13.25


>< Tru9I
>< MseI
> < RmaI
> < Esp4I
> < MaeI
>< Eco57I
>< AluI
> < AflII
>< AluI
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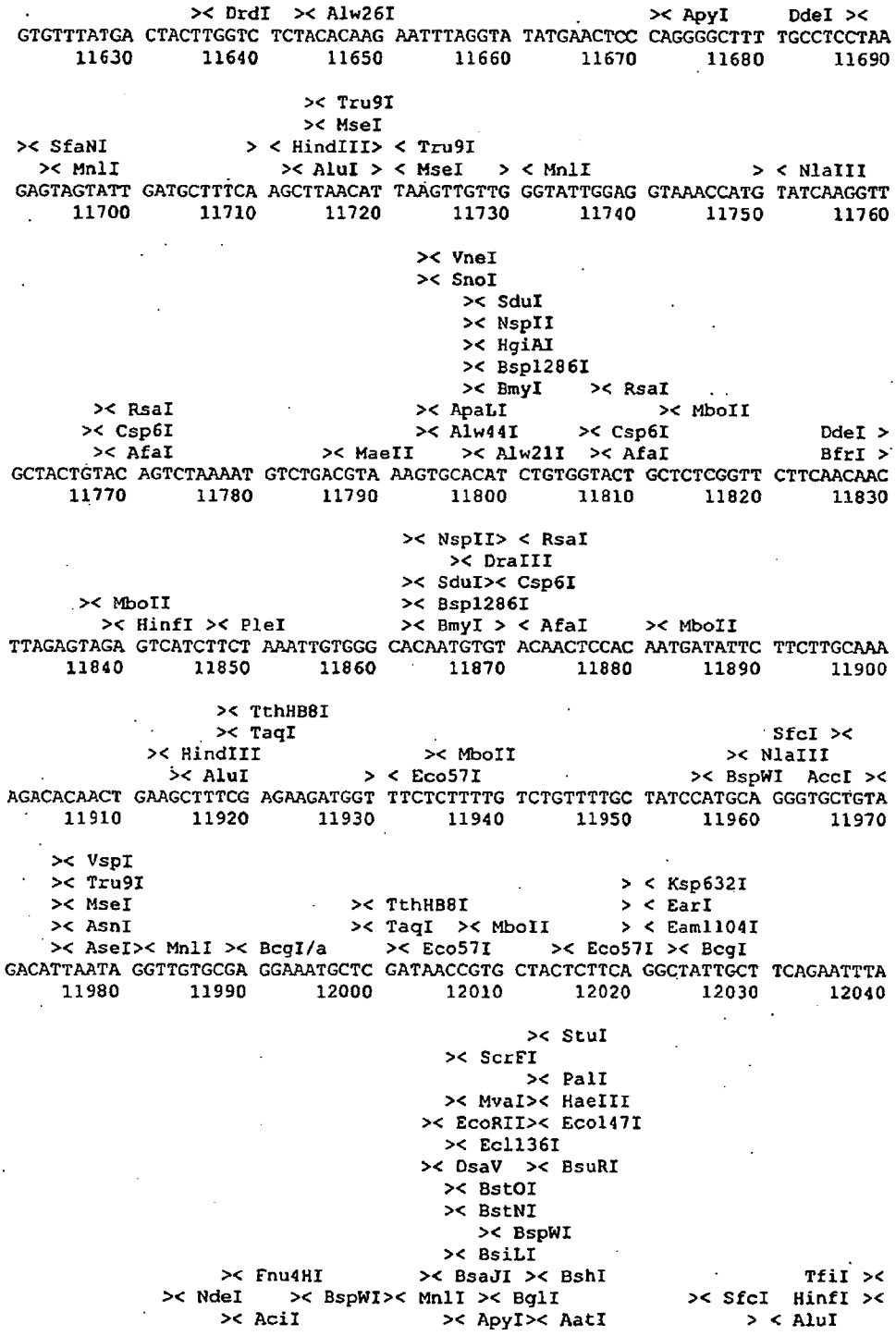
11210 11220 11230 11240 11250 11260 11270
>< RmaI
>< MaeII
>< MaeI
> < NlaIII >< SfaNI >< Fnu4HI
>< BspHI >< AluI >< BbvI >< AflIII
TATTCTCATG ACAGCTCGCA CTGTTTATGA TGATGCTGCT AGACGTGTTT GGACACTGAT GAATGTCATT
11280 11290 11300 11310 11320 11330 11340
>< Sau96I
>< PalI
>< NspIV
>< NlaIII
>< HaeIII
>< Sau3AI > < DdeI
>< NdeII >< Cfr13I
>< MboI >< BsuRI
>< DpnII >< BsiZI
>< DpnI >< BshI
>< Bspl43I > < BfrI
>< AccI >< BspAI>< AluI >< AsuI
ACACTTGTTT ACAAAGTCTA CTATGGTAAT GCTTTAGATC AAGCTATTTC CATGTGGGCC TTAGTTATTT
11350 11360 11370 11380 11390 11400 11410
>< RmaI
>< NlaIII
>< MaeI>< SfcI
>< MaeIII >< MnlI >< MaeIII >< AluI>< AluI
CTGTAACCTC TAACTATTCT GGTGTCGTTA CGACTATCAT GTTTTTAGCT AGAGCTATAG TGTTTGTGTG
11420 11430 11440 11450 11460 11470 11480
DdeI >
>< BsrI >< NlaIII BfrI >
TGTTGAGTAT TACCCATTGT TATTTATTAC TGGCAACACC TTACAGTGTA TCATGCTTGT TTATTGTTTC
11490 11500 11510 11520 11530 11540 11550
>< PalI
>< HaeIII
>< Fnu4HI >< BsuRI
>< BbvI >< Fnu4HI >< BspWI
>< BbvI >< BspWI >< BshI >< Eco57I >< MaeIII
TTAGGCTATT GTTGCTGCTG CTACTTTGGC CTTTTCTGTT TACTCAACCG TTACTTCAGG CTTACTCTTG
11560 11570 11580 11590 11600 11610 11620
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< Eco31I >< BsiLI
>< BsmAI > < BsaJI
>< BsaI >< BsaJI

FIGURE 13.26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11630 11640 11650 11660 11670 11680 11690
>< Tru9I
>< MseI
>< SfaNI > < HindIII> < Tru9I
>< MnlI >< AluI > < MseI > < MnlI > < NlaIII
GAGTAGTATT GATGCTTTCA AGCTTAACAT TAAGTTGTTG GGTATTGGAG GTAAACCATG TATCAAGGTT
11700 11710 11720 11730 11740 11750 11760
>< VneI
>< SnoI
>< SduI
>< NspII
>< HgiAI
>< Bsp1286I
>< BmyI >< RsaI
>< RsaI >< ApaLI >< MboII
>< Csp6I >< Alw44I >< Csp6I DdeI >
>< AfaI >< MaeII >< Alw21I >< AfaI BfrI >
GCTACTGTAC AGTCTAAAAT GTCTGACGTA AAGTGCACAT CTGTGGTACT GCTCTCGGTT CTTCAACAAC
11770 11780 11790 11800 11810 11820 11830
>< NspII> < RsaI
>< DraIII
>< SduI>< Csp6I
>< MboII >< Bsp1286I
>< HinfI >< PleI >< BmyI > < AfaI >< MboII
TTAGAGTAGA GTCATCTTCT AAATTGTGGG CACAATGTGT ACAACTCCAC AATGATATTC TTCTTGCAAA
11840 11850 11860 11870 11880 11890 11900
>< TthHB8I
>< TaqI
>< HindIII >< MboII SfcI ><
>< AluI > < Eco57I >< NlaIII
>< BspWI AccI ><
AGACACAACT GAAGCTTTCG AGAAGATGGT TTCTCTTTTG TCTGTTTTGC TATCCATGCA GGGTGCTGTA
11910 11920 11930 11940 11950 11960 11970
>< VspI
>< Tru9I > < Ksp632I
>< MseI >< TthHB8I > < EarI
>< AsnI >< TaqI >< MboII > < Eam1104I
>< AseI>< MnlI >< BcgI/a >< Eco57I >< Eco57I >< BcgI
GACATTAATA GGTTGTGCGA GGAAATGCTC GATAACCGTG CTACTCTTCA GGCTATTGCT TCAGAATTTA
11980 11990 12000 12010 12020 12030 12040
>< StuI
>< ScrFI
>< PalI
>< MvaI>< HaeIII
>< EcoRII>< Eco147I
>< Ecl136I
>< DsaV >< BsuRI
>< BstOI
>< BstNI
>< BspWI
>< BsiLI
>< Fnu4HI >< BsaJI >< BshI TfiI ><
>< NdeI >< BspWI>< MnlI >< BglI >< SfcI HinfI ><
>< AciI >< ApyI>< AatI > < AluI

FIGURE 13. 27

```
GTTCTTTACC ATCATATGCC GCTTATGCCA CTGCCCAGGA GGCCTATGAG CAGGCTGTAG CTAATGGTGA
    12050      12060      12070      12080      12090      12100      12110

       >< XmnI         >< Tru9I                        >< SfaNI
      >< HphI          >< MseI                      >< DdeI
       >< Asp700I       >< Eco57I                      >< BbvI Fnu4HI ><
TTCTGAAGTC GTTCTCAAAA AGTTAAAGAA ATCTTTGAAT GTGGCTAAAT CTGAGTTTGA CCGTGATGCT
    12120      12130      12140      12150      12160      12170      12180

                                                        XhoII ><
                                                       Sau3AI ><
                                                       NdeII ><
                                                              MnlI >
                                                           >< MnlI
                                                             >< MflI
                                  > < Sau3AI                 >< MboI
                                  > < NdeII                DpnII ><
                                  > < MboI                    DpnI ><
                                  > < DpnII                    DdeI ><
                                     >< DpnI                 BstYI ><
                                       >< BspWI           >< RsaIBspAI ><
                                  > < BspAI              >< Csp6IBsp143I ><
    >< NlaIII                        >< Bsp143I          >< AfaIBglII ><
GCCATGCAAC GCAAGTTGGA AAAGATGGCA GATCAGGCTA TGACCCAAAT GTACAAACAG GCAAGATCTG
    12190      12200      12210      12220      12230      12240      12250

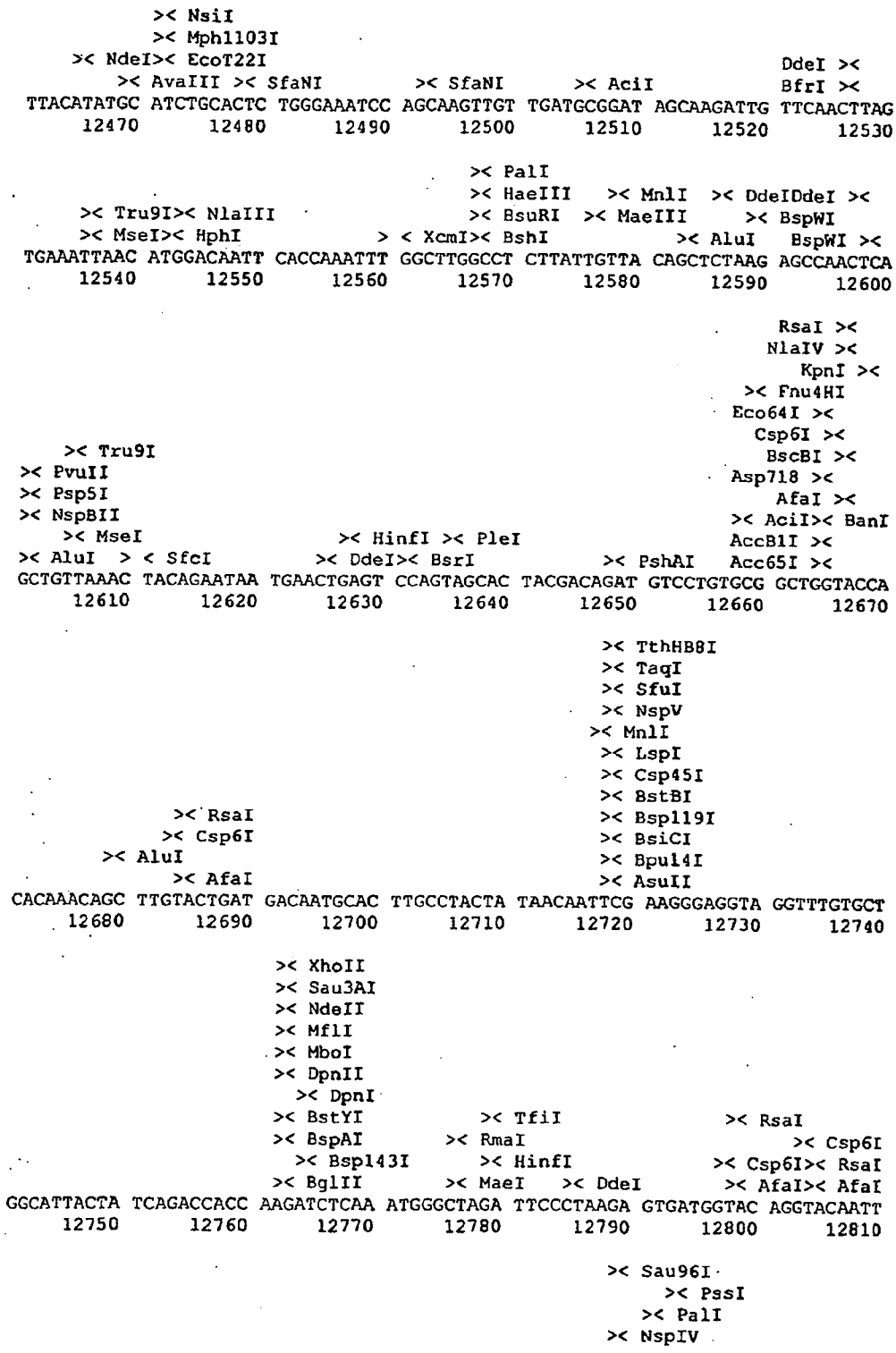
                     >< SpeI                      >< Ksp632I   > < HindIII
                      >< RmaI                           >< DdeI  >< SfaNI
                 >< MaeIII          >< MboII      >< Eam1104I >< BspWI
                      >< MaeI          >< BspWI    >< EarI>< BfrI  >< AluI
AGGACAAGAG GGCAAAAGTA ACTAGTGCTA TGCAAACAAT GCTCTTCACT ATGCTTAGGA AGCTTGATAA
    12260      12270      12280      12290      12300      12310      12320

                                 >< ThaI
                                 >< MvnI
                              >< HinP1I
                              >< Hin6I
                                 >< HhaI
                                 >< CfoI
                                 >< BstUI
       >< Tru9I                  >< Bsp50I
       >< MseI                   >< AccII                              SfcI ><
TGATGCACTT AACAACATTA TCAACAATGC GCGTGATGGT TGTGTTCCAC TCAACATCAT ACCATTGACT
    12330      12340      12350      12360      12370      12380      12390

                                       >< RsaI
                                       >< NlaIV
                                    >< Eco64I
                                     >< Csp6I
                                  >< BslI
                                  >< BslYI>< KpnI
                                     >< BscBI
                                  >< BanI
                                  >< Asp718
                 >< NlaIII          >< AfaI
                >< BstXI            >< AccB1I                    >< MaeIII
   >< Fnu4HI    >< BbvI             >< Acc65I                      BsgI ><
ACAGCAGCCA AACTCATGGT TGTTGTCCCT GATTATGGTA CCTACAAGAA CACTTGTGAT GGTAACACCT
    12400      12410      12420      12430      12440      12450      12460

         >< Zsp2I
      >< Ppu10I
```

FIGURE 13.28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DdeI ><
>< AvaIII >< SfaNI >< SfaNI >< AciI BfrI ><
TTACATATGC ATCTGCACTC TGGGAAATCC AGCAAGTTGT TGATGCGGAT AGCAAGATTG TTCAACTTAG
12470 12480 12490 12500 12510 12520 12530
>< PalI
>< HaeIII >< MnlI >< DdeIDdeI ><
>< Tru9I>< NlaIII >< BsuRI >< MaeIII >< BspWI
>< MseI>< HphI > < XcmI>< BshI >< AluI BspWI ><
TGAAATTAAC ATGGACAATT CACCAAATTT GGCTTGGCCT CTTATTGTTA CAGCTCTAAG AGCCAACTCA
12540 12550 12560 12570 12580 12590 12600
RsaI ><
NlaIV ><
KpnI ><
>< Fnu4HI
Eco64I ><
Csp6I ><
>< Tru9I BscBI ><
>< PvuII Asp718 ><
>< Psp5I AfaI ><
>< NspBII >< AciI>< BanI
>< MseI AccB1I ><
>< AluI > < SfcI >< HinfI >< PleI >< DdeI>< BsrI >< PshAI Acc65I ><
GCTGTTAAAC TACAGAATAA TGAACTGAGT CCAGTAGCAC TACGACAGAT GTCCTGTGCG GCTGGTACCA
12610 12620 12630 12640 12650 12660 12670
>< TthHB8I
>< TaqI
>< SfuI
>< NspV
>< MnlI
>< LspI
>< Csp45I
>< BstBI
>< RsaI >< Bsp119I
>< Csp6I >< BsiCI
>< AluI >< Bpu14I
>< AfaI >< AsuII
CACAAACAGC TTGTACTGAT GACAATGCAC TTGCCTACTA TAACAATTCG AAGGGAGGTA GGTTTGTGCT
12680 12690 12700 12710 12720 12730 12740
>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< DpnII
>< DpnI
>< BstYI >< TfiI >< RsaI
>< BspAI >< RmaI >< Csp6I
>< Bsp143I >< HinfI >< Csp6I>< RsaI
>< BglII >< MaeI >< DdeI >< AfaI>< AfaI
GGCATTACTA TCAGACCACC AAGATCTCAA ATGGGCTAGA TTCCCTAAGA GTGATGGTAC AGGTACAATT
12750 12760 12770 12780 12790 12800 12810
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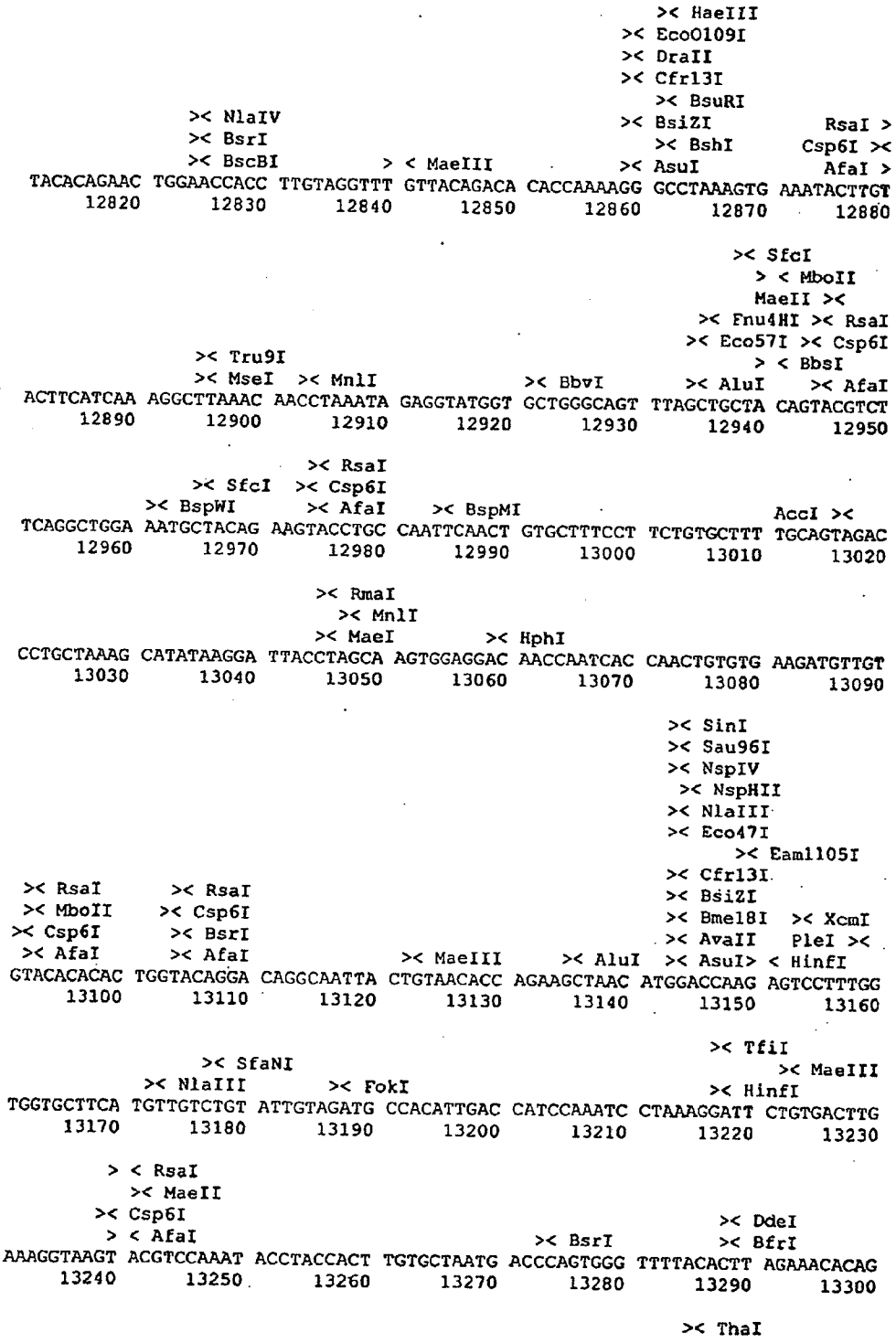
>< NspIV

FIGURE 13.29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>< DraII
>< Cfr13I
>< BsuRI
>< NlaIV
>< BsrI
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>< BshI
RsaI >
Csp6I ><
> < MaeIII
>< AsuI
AfaI >
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12820 12830 12840 12850 12860 12870 12880
>< SfcI
> < MboII
MaeII ><
>< Fnu4HI >< RsaI
>< Eco57I >< Csp6I
> < BbsI
>< Tru9I
>< MseI >< MnlI
>< BbvI
>< AluI
>< AfaI
ACTTCATCAA AGGCTTAAAC AACCTAAATA GAGGTATGGT GCTGGGCAGT TTAGCTGCTA CAGTACGTCT
12890 12900 12910 12920 12930 12940 12950
>< RsaI
>< SfcI >< Csp6I
>< BspWI
>< AfaI
>< BspMI
AccI ><
TCAGGCTGGA AATGCTACAG AAGTACCTGC CAATTCAACT GTGCTTTCCT TCTGTGCTTT TGCAGTAGAC
12960 12970 12980 12990 13000 13010 13020
>< RmaI
>< MnlI
>< MaeI
>< HphI
CCTGCTAAAG CATATAAGGA TTACCTAGCA AGTGGAGGAC AACCAATCAC CAACTGTGTG AAGATGTTGT
13030 13040 13050 13060 13070 13080 13090
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< NlaIII
>< Eco47I
>< Eam1105I
>< Cfr13I
>< RsaI
>< MboII
>< Csp6I
>< AfaI
>< RsaI
>< Csp6I
>< BsrI
>< AfaI
>< BsiZI
>< Bme18I
>< XcmI
>< AvaII
PleI ><
>< MaeIII
>< AluI
>< AsuI
> < HinfI
GTACACACAC TGGTACAGGA CAGGCAATTA CTGTAACACC AGAAGCTAAC ATGGACCAAG AGTCCTTTGG
13100 13110 13120 13130 13140 13150 13160
>< TfiI
>< MaeIII
>< SfaNI
>< NlaIII
>< FokI
>< HinfI
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13170 13180 13190 13200 13210 13220 13230
> < RsaI
>< MaeII
>< Csp6I
> < AfaI
>< BsrI
>< DdeI
>< BfrI
AAAGGTAAGT ACGTCCAAAT ACCTACCACT TGTGCTAATG ACCCAGTGGG TTTTACACTT AGAAACACAG
13240 13250 13260 13270 13280 13290 13300
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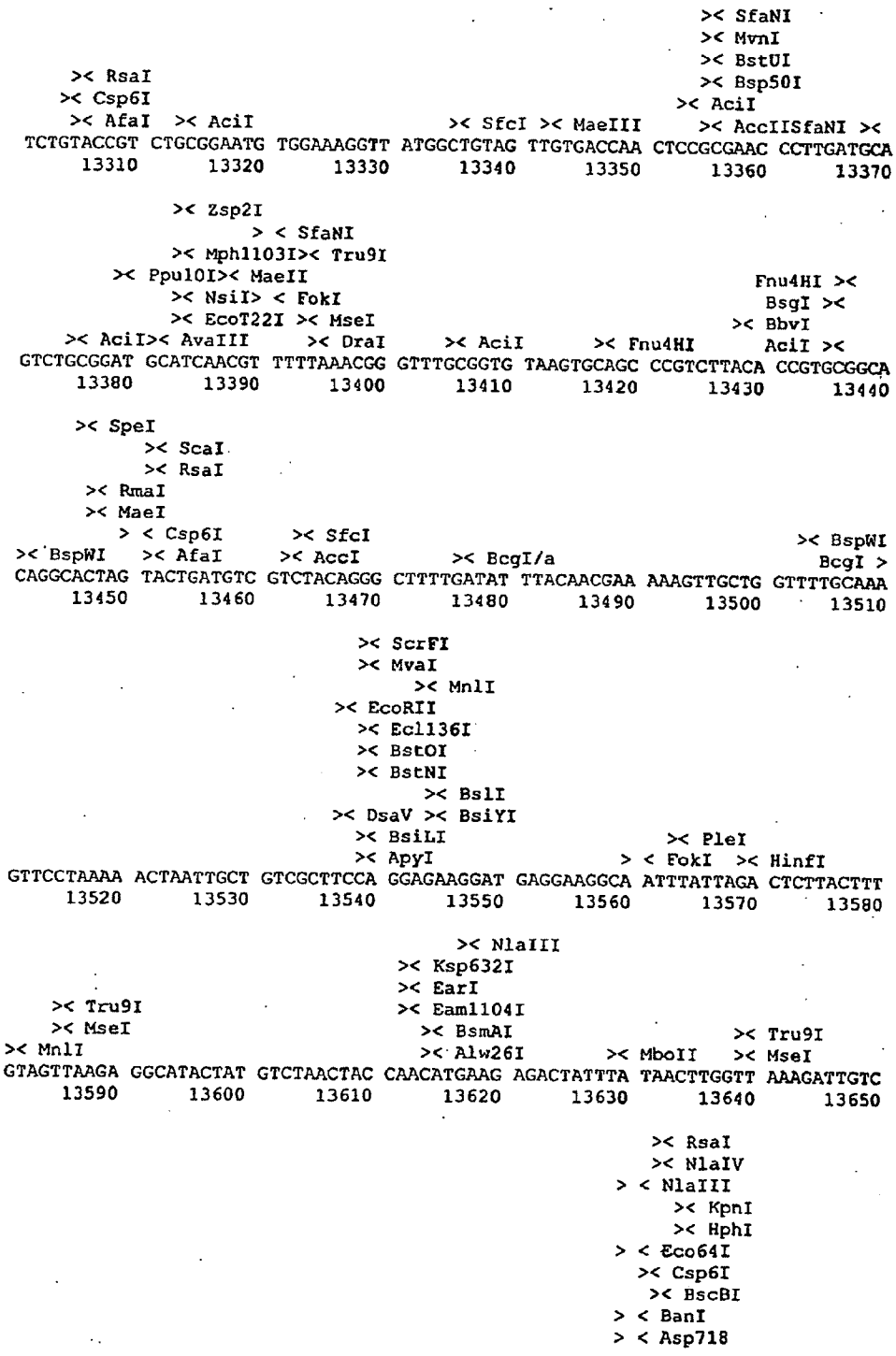
FIGURE 13.30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>< BstUI
>< Bsp50I
>< AciI
>< RsaI
>< Csp6I
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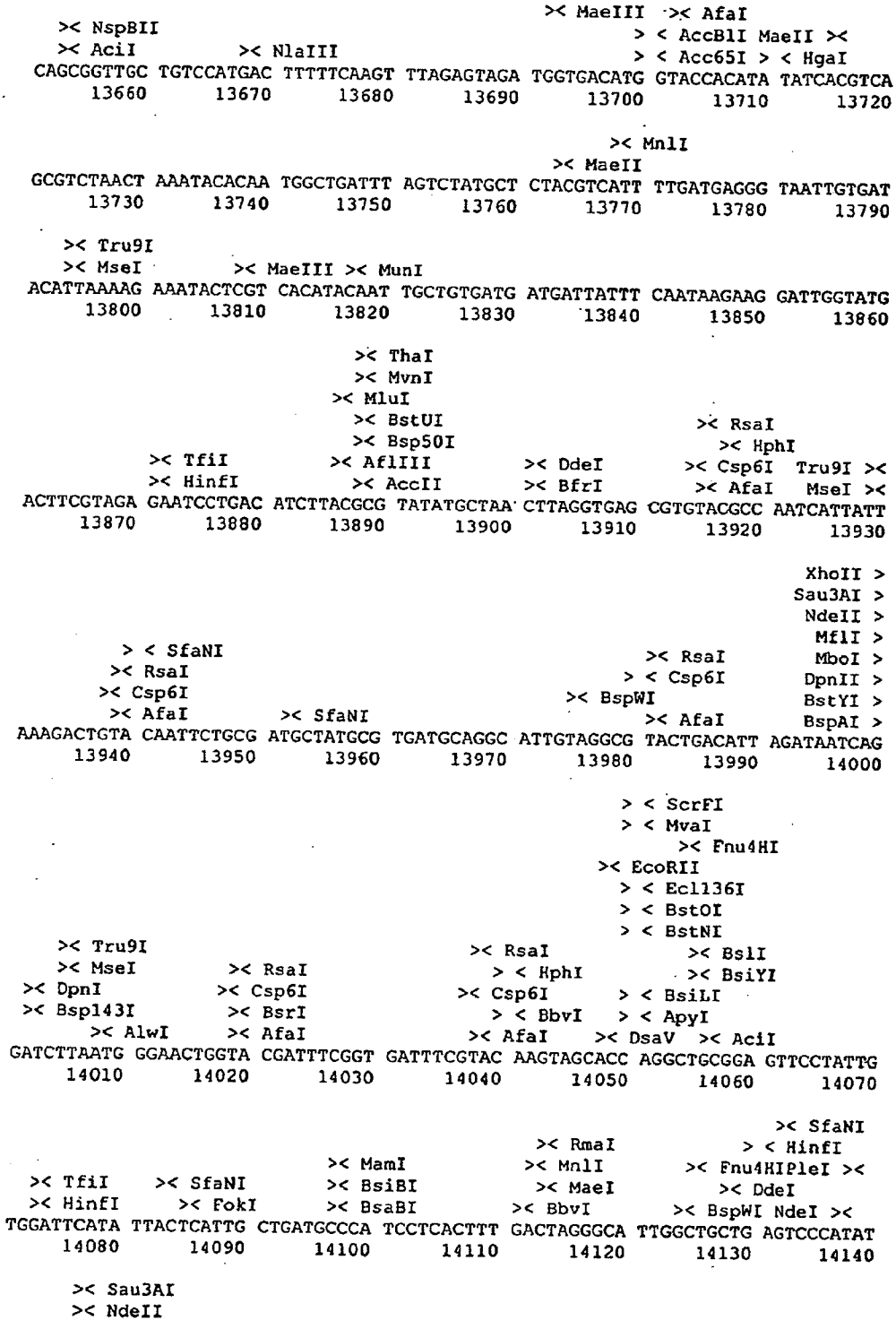
TCTGTACCGT CTGCGGAATG TGGAAAGGTT ATGGCTGTAG TTGTGACCAA CTCCGCGAAC CCTTGATGCA
13310 13320 13330 13340 13350 13360 13370
>< Zsp2I
> < SfaNI
>< Mph1103I>< Tru9I
>< Ppu10I>< MaeII
Fnu4HI ><
BsgI ><
>< BbvI
>< NsiI> < FokI
>< EcoT22I >< MseI
>< AciI>< AvaIII >< DraI >< AciI >< Fnu4HI AciI ><
GTCTGCGGAT GCATCAACGT TTTTAAACGG GTTTGCGGTG TAAGTGCAGC CCGTCTTACA CCGTGCGGCA
13380 13390 13400 13410 13420 13430 13440
>< SpeI
>< ScaI
>< RsaI
>< RmaI
>< MaeI
> < Csp6I >< SfcI >< BspWI
>< BspWI >< AfaI >< AccI >< BcgI/a BcgI >
CAGGCACTAG TACTGATGTC GTCTACAGGG CTTTTGATAT TTACAACGAA AAAGTTGCTG GTTTTGCAAA
13450 13460 13470 13480 13490 13500 13510
>< ScrFI
>< MvaI
>< MnlI
>< EcoRII
>< Ecl136I
>< BstOI
>< BstNI
>< BslI
>< DsaV >< BsiYI
>< BsiLI >< PleI
>< ApyI > < FokI >< HinfI
GTTCCTAAAA ACTAATTGCT GTCGCTTCCA GGAGAAGGAT GAGGAAGGCA ATTTATTAGA CTCTTACTTT
13520 13530 13540 13550 13560 13570 13580
>< NlaIII
>< Ksp632I
>< EarI
>< Eam1104I
>< Tru9I
>< MseI
>< BsmAI >< Tru9I
>< MnlI >< Alw26I >< MboII >< MseI
GTAGTTAAGA GGCATACTAT GTCTAACTAC CAACATGAAG AGACTATTTA TAACTTGGTT AAAGATTGTC
13590 13600 13610 13620 13630 13640 13650
>< RsaI
>< NlaIV
> < NlaIII
>< KpnI
>< HphI
> < Eco64I
>< Csp6I
>< BscBI
> < BanI
> < Asp718

FIGURE 13.31


>< MaeIII >< AfaI
>< NspBII
>< AciI
>< NlaIII
> < AccBlI MaeII ><
> < Acc65I > < HgaI
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13660 13670 13680 13690 13700 13710 13720
>< MnlI
>< MaeII
GCGTCTAACT AAATACACAA TGGCTGATTT AGTCTATGCT CTACGTCATT TTGATGAGGG TAATTGTGAT
13730 13740 13750 13760 13770 13780 13790
>< Tru9I
>< MseI
>< MaeIII >< MunI
ACATTAAAAG AAATACTCGT CACATACAAT TGCTGTGATG ATGATTATTT CAATAAGAAG GATTGGTATG
13800 13810 13820 13830 13840 13850 13860
>< ThaI
>< MvnI
>< MluI
>< BstUI
>< Bsp50I
>< RsaI
>< HphI
>< TfiI
>< HinfI
>< AflIII
>< AccII
>< DdeI
>< BfrI
>< Csp6I Tru9I ><
>< AfaI MseI ><
ACTTCGTAGA GAATCCTGAC ATCTTACGCG TATATGCTAA CTTAGGTGAG CGTGTACGCC AATCATTATT
13870 13880 13890 13900 13910 13920 13930
XhoII >
Sau3AI >
NdeII >
MflI >
> < SfaNI
>< RsaI
>< Csp6I
>< AfaI
>< SfaNI
>< RsaI
> < Csp6I
>< BspWI
>< AfaI
MboI >
DpnII >
BstYI >
BspAI >
AAAGACTGTA CAATTCTGCG ATGCTATGCG TGATGCAGGC ATTGTAGGCG TACTGACATT AGATAATCAG
13940 13950 13960 13970 13980 13990 14000
> < ScrFI
> < MvaI
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>< EcoRII
> < Ecl136I
> < BstOI
> < BstNI
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>< MseI
>< DpnI
>< Bsp143I
>< AlwI
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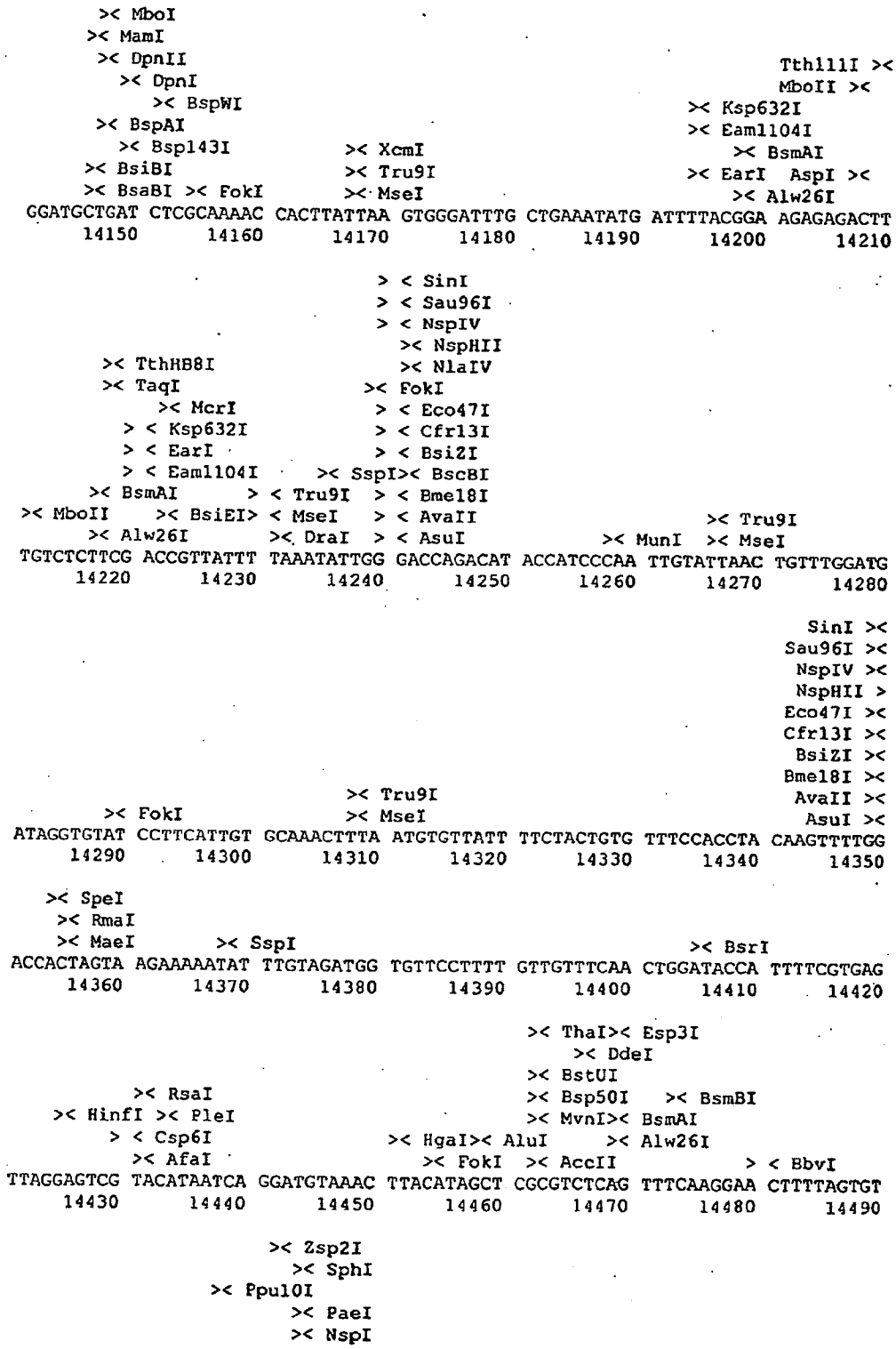
>< AfaI
>< RsaI
> < HphI
>< Csp6I
> < BbvI
>< AfaI
>< BslI
>< BsiYI
> < BsiLI
> < ApyI
>< DsaV >< AciI
GATCTTAATG GGAACTGGTA CGATTTCGGT GATTTCGTAC AAGTAGCACC AGGCTGCGGA GTTCCTATTG
14010 14020 14030 14040 14050 14060 14070
>< SfaNI
>< RmaI
> < HinfI
>< MamI
>< MnlI
>< Fnu4HIPleI ><
>< TfiI
>< HinfI
>< SfaNI
>< FokI
>< BsiBI
>< BsaBI
>< MaeI
>< DdeI
>< BbvI
>< BspWI NdeI ><
TGGATTCATA TTACTCATTG CTGATGCCCA TCCTCACTTT GACTAGGGCA TTGGCTGCTG AGTCCCATAT
14080 14090 14100 14110 14120 14130 14140
>< Sau3AI
>< NdeII

FIGURE 13.32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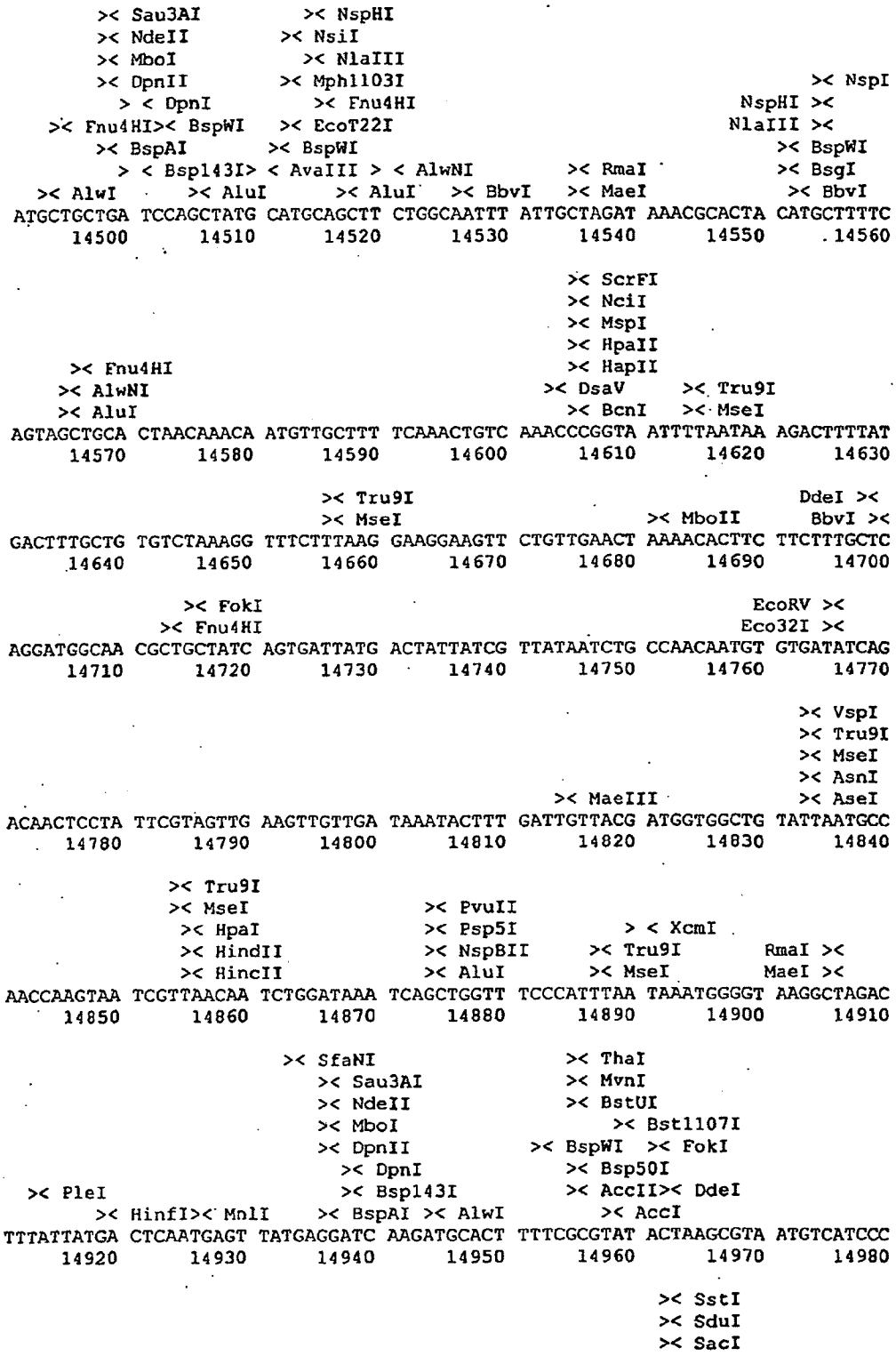
>< MamI
>< DpnII
>< DpnI
>< BspWI
>< BspAI
>< Bsp143I
>< BsiBI
>< BsaBI >< FokI
>< XcmI
>< Tru9I
>< MseI
Tth111I ><
MboII ><
>< Ksp632I
>< Eam1104I
>< BsmAI
>< EarI AspI ><
>< Alw26I
GGATGCTGAT CTCGCAAAAC CACTTATTAA GTGGGATTTG CTGAAATATG ATTTTACGGA AGAGAGACTT
14150 14160 14170 14180 14190 14200 14210
> < SinI
> < Sau96I
> < NspIV
>< NspHII
>< NlaIV
>< FokI
> < Eco47I
> < Cfr13I
> < BsiZI
>< SspI>< BscBI
> < Bme18I
> < AvaII
> < AsuI
>< MunI
>< TthHB8I
>< TaqI
>< McrI
> < Ksp632I
> < EarI
> < Eam1104I
>< BsmAI
> < Tru9I
> < MseI
>< DraI
>< MboII
>< BsiEI>
>< Alw26I
>< Tru9I
>< MseI
TGTCTCTTCG ACCGTTATTT TAAATATTGG GACCAGACAT ACCATCCCAA TTGTATTAAC TGTTTGGATG
14220 14230 14240 14250 14260 14270 14280
SinI ><
Sau96I ><
NspIV ><
NspHII >
Eco47I ><
Cfr13I ><
BsiZI ><
Bme18I ><
AvaII ><
AsuI ><
>< Tru9I
>< MseI
>< FokI
ATAGGTGTAT CCTTCATTGT GCAAACTTTA ATGTGTTATT TTCTACTGTG TTTCCACCTA CAAGTTTTGG
14290 14300 14310 14320 14330 14340 14350
>< SpeI
>< RmaI
>< MaeI
>< SspI
>< BsrI
ACCACTAGTA AGAAAAATAT TTGTAGATGG TGTTCCTTTT GTTGTTTCAA CTGGATACCA TTTTCGTGAG
14360 14370 14380 14390 14400 14410 14420
>< ThaI>< Esp3I
>< DdeI
>< BstUI
>< Bsp50I
>< MvnI>< BsmAI
>< BsmBI
>< RsaI
>< HinfI >< PleI
> < Csp6I
>< AfaI
>< HgaI>< AluI
>< Alw26I
>< FokI
>< AccII
> < BbvI
TTAGGAGTCG TACATAATCA GGATGTAAAC TTACATAGCT CGCGTCTCAG TTTCAAGGAA CTTTTAGTGT
14430 14440 14450 14460 14470 14480 14490
>< Zsp2I
>< SphI
>< Ppu10I
>< PaeI
>< NspI

FIGURE 13.33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NspHI ><
>< Fnu4HI>< BspWI
>< EcoT22I
NlaIII ><
>< BspAI
>< BspWI
>< BspWI
> < Bsp143I> < AvaIII > < AlwNI
>< RmaI
>< BsgI
>< AlwI
>< AluI
>< AluI
>< BbvI
>< MaeI
>< BbvI
ATGCTGCTGA TCCAGCTATG CATGCAGCTT CTGGCAATTT ATTGCTAGAT AAACGCACTA CATGCTTTTC
14500 14510 14520 14530 14540 14550 14560
>< ScrFI
>< NciI
>< MspI
>< HpaII
>< Fnu4HI
>< HapII
>< AlwNI
>< DsaV
>< Tru9I
>< AluI
>< BcnI
>< MseI
AGTAGCTGCA CTAACAAACA ATGTTGCTTT TCAAACTGTC AAACCCGGTA ATTTTAATAA AGACTTTTAT
14570 14580 14590 14600 14610 14620 14630
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DdeI ><
>< MseI
>< MboII
BbvI ><
GACTTTGCTG TGTCTAAAGG TTTCTTTAAG GAAGGAAGTT CTGTTGAACT AAAACACTTC TTCTTTGCTC
14640 14650 14660 14670 14680 14690 14700
>< FokI
EcoRV ><
>< Fnu4HI
Eco32I ><
AGGATGGCAA CGCTGCTATC AGTGATTATG ACTATTATCG TTATAATCTG CCAACAATGT GTGATATCAG
14710 14720 14730 14740 14750 14760 14770
>< VspI
>< Tru9I
>< MseI
>< AsnI
>< MaeIII
>< AseI
ACAACTCCTA TTCGTAGTTG AAGTTGTTGA TAAATACTTT GATTGTTACG ATGGTGGCTG TATTAATGCC
14780 14790 14800 14810 14820 14830 14840
>< Tru9I
>< MseI
>< PvuII
>< HpaI
>< Psp5I
> < XcmI
>< HindII
>< NspBII
>< Tru9I
RmaI ><
>< HincII
>< AluI
>< MseI
MaeI ><
AACCAAGTAA TCGTTAACAA TCTGGATAAA TCAGCTGGTT TCCCATTTAA TAAATGGGGT AAGGCTAGAC
14850 14860 14870 14880 14890 14900 14910
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>< ThaI
>< Sau3AI
>< MvnI
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>< BstUI
>< MboI
>< Bst1107I
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>< BspWI >< FokI
>< DpnI
>< Bsp50I
>< PleI
>< Bsp143I
>< AccII><DdeI
>< HinfI>< MnlI
>< BspAI >< AlwI
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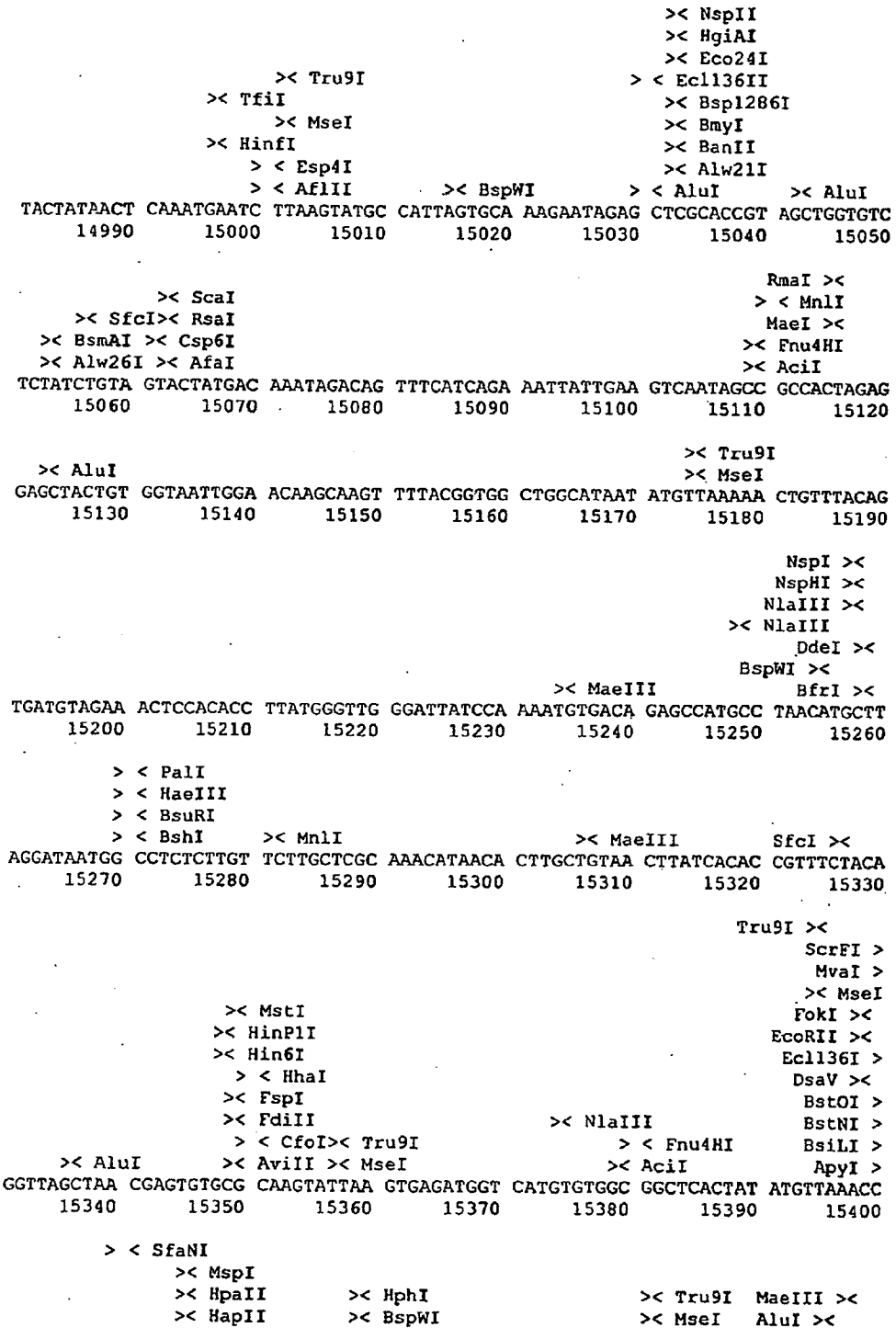
>< SstI
>< SduI
>< SacI

FIGURE 13.34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>< BanII
> < Esp4I
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> < AluI
>< AluI
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>< ScaI
> < MnlI
>< SfcI>< RsaI
MaeI ><
>< BsmAI >< Csp6I
>< Fnu4HI
>< Alw26I >< AfaI
>< AciI
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15060 15070 15080 15090 15100 15110 15120
>< Tru9I
>< AluI
>< MseI
GAGCTACTGT GGTAATTGGA ACAAGCAAGT TTTACGGTGG CTGGCATAAT ATGTTAAAAA CTGTTTACAG
15130 15140 15150 15160 15170 15180 15190
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NspHI ><
NlaIII ><
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DdeI ><
BspWI ><
>< MaeIII
BfrI ><
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> < PalI
> < HaeIII
> < BsuRI
> < BshI
>< MnlI
>< MaeIII
SfcI ><
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Tru9I ><
ScrFI >
MvaI >
>< MseI
>< MstI
FokI ><
>< HinP1I
EcoRII ><
>< Hin6I
Ecl136I >
> < HhaI
DsaV ><
>< FspI
BstOI >
>< FdiII
>< NlaIII
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BsiLI >
>< AluI
>< AviII >< MseI
>< AciI
ApyI >
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>< HphI
>< Tru9I MaeIII ><
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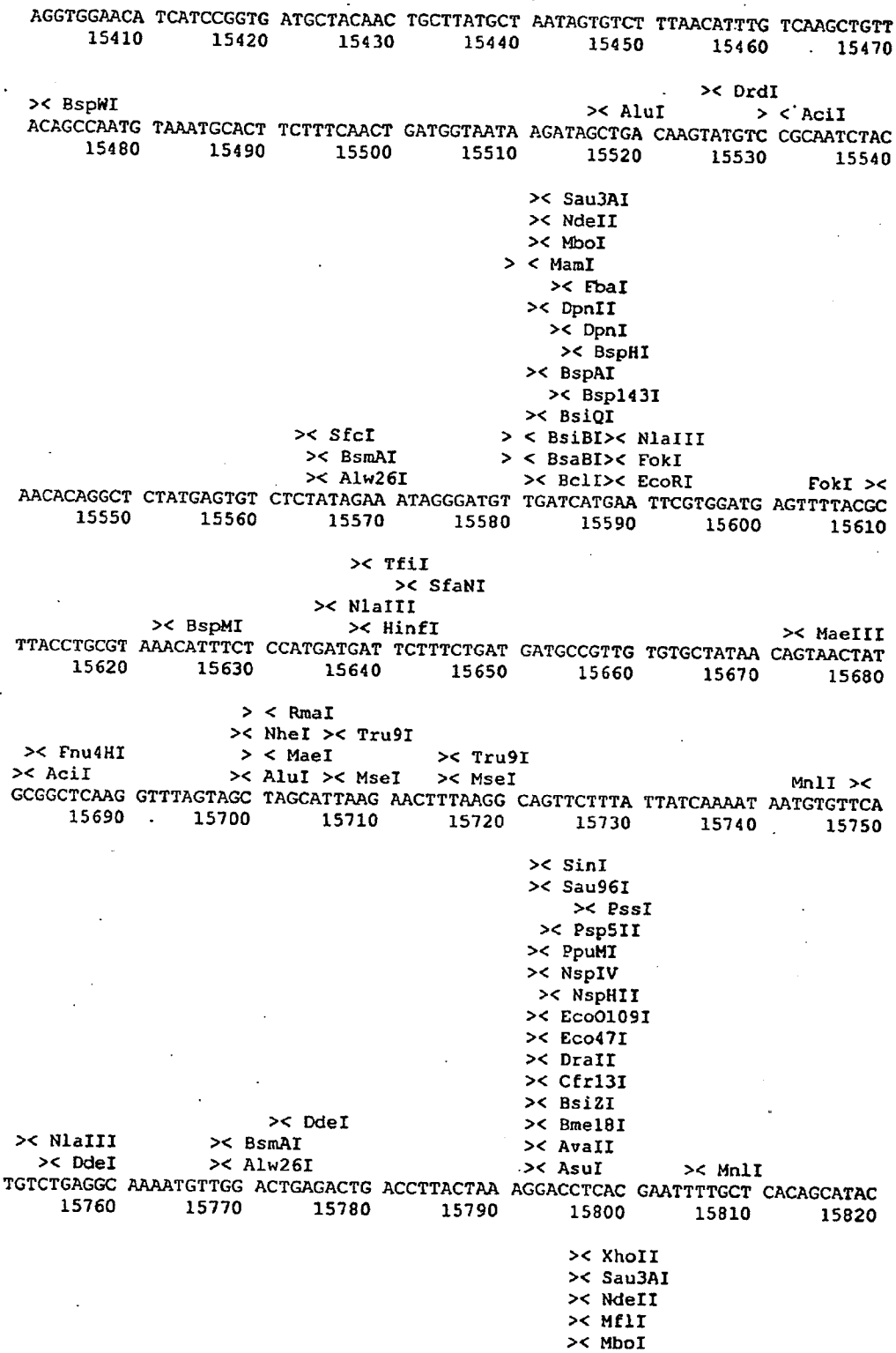
FIGURE 13.35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> < MamI
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>< DpnII
>< DpnI
>< BspHI
>< BspAI
>< Bsp143I
>< BsiQI
>< SfcI
> < BsiBI>< NlaIII
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> < BsaBI>< FokI
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FokI ><
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>< NlaIII
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>< HinfI
>< MaeIII
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15620 15630 15640 15650 15660 15670 15680
> < RmaI
>< NheI >< Tru9I
>< Fnu4HI
> < MaeI
>< Tru9I
>< AciI
>< AluI >< MseI
>< MseI
MnlI ><
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15690 15700 15710 15720 15730 15740 15750
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>< Psp5II
>< PpuMI
>< NspIV
>< NspHII
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>< DraII
>< Cfr13I
>< BsiZI
>< DdeI
>< Bme18I
>< NlaIII
>< BsmAI
>< AvaII
>< DdeI
>< Alw26I
>< AsuI
>< MnlI
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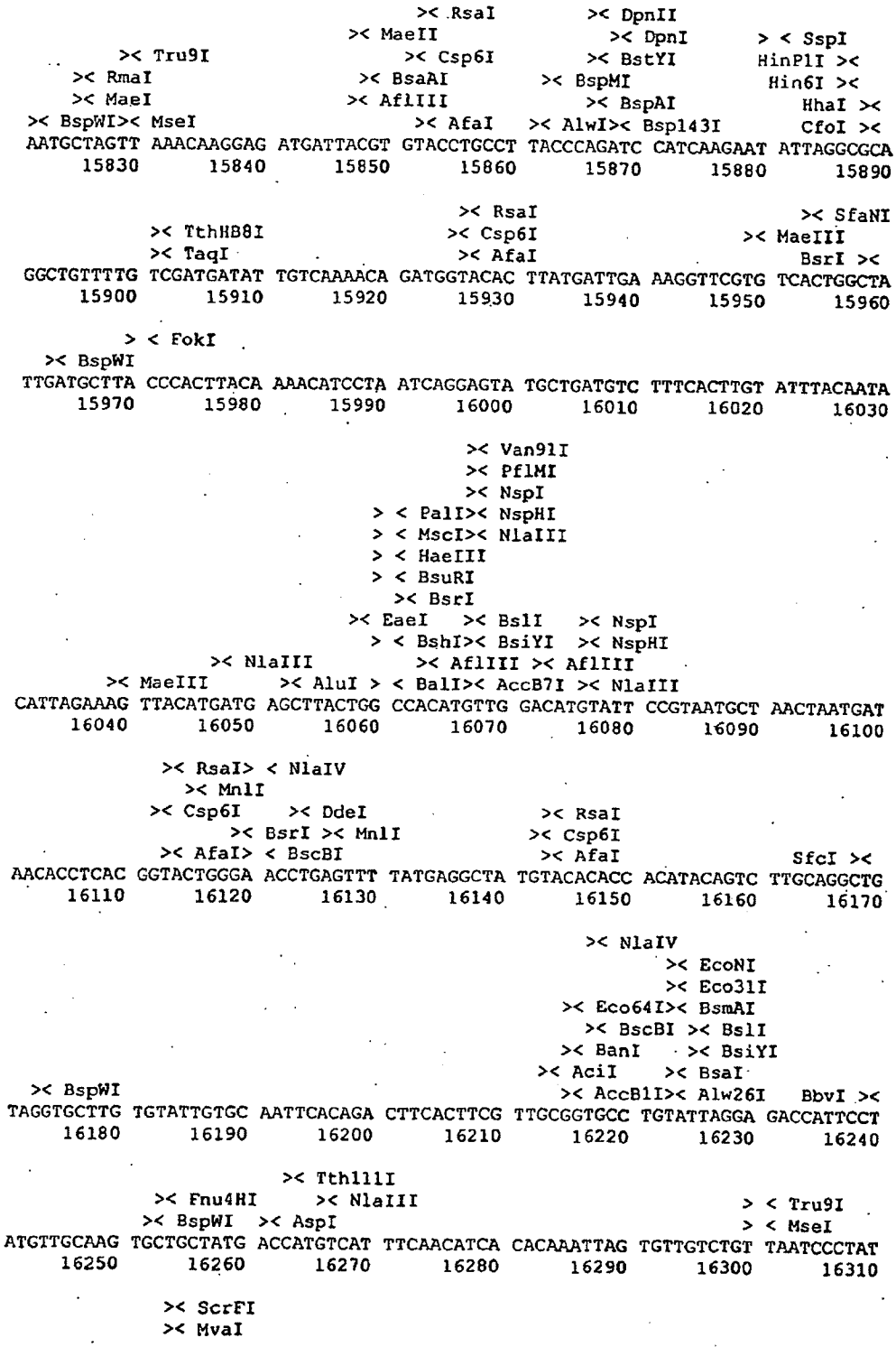
>< Sau3AI
>< NdeII
>< MflI
>< MboI

FIGURE 13. 36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>< MaeII >< DpnI > < SspI
>< Tru9I >< Csp6I >< BstYI HinP1I ><
>< RmaI >< BsaAI >< BspMI Hin6I ><
>< MaeI >< AflIII >< BspAI HhaI ><
>< BspWI>< MseI >< AfaI >< AlwI>< Bsp143I CfoI ><
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>< RsaI >< SfaNI
>< TthHB8I >< Csp6I >< MaeIII
>< TaqI >< AfaI BsrI ><
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15900 15910 15920 15930 15940 15950 15960
> < FokI
>< BspWI
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15970 15980 15990 16000 16010 16020 16030
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> < PalI>< NspHI
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> < BsuRI
>< BsrI
>< EaeI >< BslI >< NspI
> < BshI>< BsiYI >< NspHI
>< NlaIII >< AflIII >< AflIII
>< MaeIII >< AluI > < BalI>< AccB7I >< NlaIII
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>< MnlI
>< Csp6I >< DdeI >< RsaI
>< BsrI >< MnlI >< Csp6I
>< AfaI> < BscBI >< AfaI SfcI ><
AACACCTCAC GGTACTGGGA ACCTGAGTTT TATGAGGCTA TGTACACACC ACATACAGTC TTGCAGGCTG
16110 16120 16130 16140 16150 16160 16170
>< NlaIV
>< EcoNI
>< Eco31I
>< Eco64I>< BsmAI
>< BscBI >< BslI
>< BanI >< BsiYI
>< AciI >< BsaI
>< BspWI >< AccB1I>< Alw26I BbvI ><
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16180 16190 16200 16210 16220 16230 16240
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>< Fnu4HI >< NlaIII > < Tru9I
>< BspWI >< AspI > < MseI
ATGTTGCAAG TGCTGCTATG ACCATGTCAT TTCAACATCA CACAAATTAG TGTTGTCTGT TAATCCCTAT
16250 16260 16270 16280 16290 16300 16310
>< ScrFI
>< MvaI

FIGURE 13.37

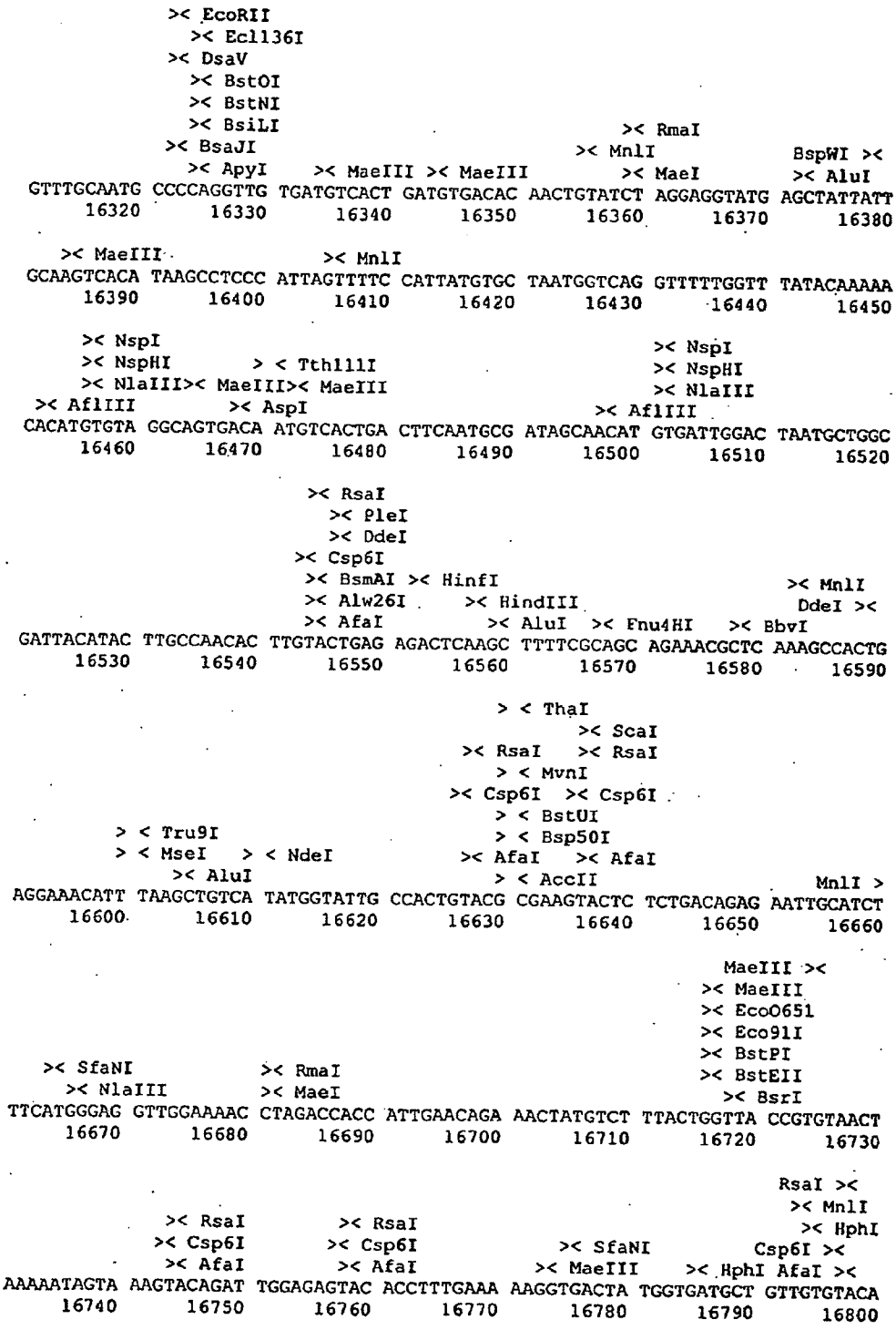

>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< BsaJI
>< ApyI
>< MaeIII >< MaeIII
>< RmaI
>< MnlI
>< MaeI
BspWI ><
>< AluI
GTTTGCAATG CCCCAGGTTG TGATGTCACT GATGTGACAC AACTGTATCT AGGAGGTATG AGCTATTATT
16320 16330 16340 16350 16360 16370 16380
>< MaeIII
>< MnlI
GCAAGTCACA TAAGCCTCCC ATTAGTTTTC CATTATGTGC TAATGGTCAG GTTTTTGGTT TATACAAAAA
16390 16400 16410 16420 16430 16440 16450
>< NspI
>< NspHI
>< NlaIII
> < Tth111I
>< MaeIII
>< AflIII
>< AspI
>< NspI
>< NspHI
>< NlaIII
>< AflIII
CACATGTGTA GGCAGTGACA ATGTCACTGA CTTCAATGCG ATAGCAACAT GTGATTGGAC TAATGCTGGC
16460 16470 16480 16490 16500 16510 16520
>< RsaI
>< PleI
>< DdeI
>< Csp6I
>< BsmAI >< HinfI
>< Alw26I
>< HindIII
>< AfaI
>< AluI
>< Fnu4HI
>< MnlI
DdeI ><
>< BbvI
GATTACATAC TTGCCAACAC TTGTACTGAG AGACTCAAGC TTTTCGCAGC AGAAACGCTC AAAGCCACTG
16530 16540 16550 16560 16570 16580 16590
> < ThaI
>< ScaI
>< RsaI
>< RsaI
> < MvnI
>< Csp6I
>< Csp6I
> < BstUI
> < Bsp50I
> < Tru9I
> < MseI
> < NdeI
>< AfaI
>< AfaI
>< AluI
> < AccII
MnlI >
AGGAAACATT TAAGCTGTCA TATGGTATTG CCACTGTACG CGAAGTACTC TCTGACAGAG AATTGCATCT
16600 16610 16620 16630 16640 16650 16660
MaeIII ><
>< MaeIII
>< Eco0651
>< Eco91I
>< BstPI
>< BstEII
>< BsrI
>< SfaNI
>< NlaIII
>< RmaI
>< MaeI
TTCATGGGAG GTTGGAAAAC CTAGACCACC ATTGAACAGA AACTATGTCT TTACTGGTTA CCGTGTAACT
16670 16680 16690 16700 16710 16720 16730
RsaI ><
>< MnlI
>< HphI
>< RsaI
>< Csp6I
>< AfaI
>< RsaI
>< Csp6I
>< AfaI
>< SfaNI
>< MaeIII
Csp6I ><
>< HphI AfaI ><
AAAAATAGTA AAGTACAGAT TGGAGAGTAC ACCTTTGAAA AAGGTGACTA TGGTGATGCT GTTGTGTACA
16740 16750 16760 16770 16780 16790 16800

FIGURE 13. 38

```
     >< RsaI                                    >< HphI
    >< Csp6I                                        >< HindII          DdeI ><
     >< AfaI                                        >< HincII          BfrI ><
GAGGTACTAC GACATACAAG TTGAATGTTG GTGATTACTT TGTGTTGACA TCTCACACTG TAATGCCACT
    16810      16820      16830      16840      16850      16860      16870

   >< VneI
   >< SnoI
      >< SduI
      >< NspII
      >< HgiAI                 > < SduI
  >< DraIII                    > < NspII
      >< Bsp1286I              > < HgiAI
      >< BmyI                >< BspWI  >< DraIII          >< RsaI
   >< ApaLI    >< RmaI        > < Bsp1286I              >< Csp6I
   >< Alw44I   >< MaeI        > < BmyI              >< BsrI
      >< Alw21I               > < Alw21I                 >< AfaI      DdeI >
TAGTGCACCT ACTCTAGTGC CACAAGAGCA CTATGTGAGA ATTACTGGCT TGTACCCAAC ACTCAACATC
    16880      16890      16900      16910      16920      16930      16940

                                                                  StyI ><
                                                                     SinI >
                                                                   Sau96I >
                                                                    NspIV >
                                                                EcoT14I ><
                                                                    Eco47I >
                                                                Eco130I ><
                                                            >< ScaI  Cfr13I >
                                                                BssT1I ><
                                                       >< SphI >< RsaI   BsiZI >
                                                       >< PaeI       BsaJI ><
                                                       >< NlaIII        Bme18I >
             >< RmaI                                   >< NspI>< Csp6I   AvaII >
             >< MaeI                                   >< NspHI>< AfaI    AsuI >
TCAGATGAGT TTTCTAGCAA TGTTGCAAAT TATCAAAAGG TCGGCATGCA AAAGTACTCT ACACTCCAAG
    16950      16960      16970      16980      16990      17000      17010

      >< ScrFI
          >< RsaI
      >< MvaI
    >< EcoRII
      >< Ecl136I
        > < Csp6I
      >< BstOI
      >< BstNI
  >< XcmI   >< BslI
>< NspHII   >< BsiYI
      >< BsiLI
      >< ApyI    >< BsrI
   >< DsaV>< AfaI  > < HinfI>< PleI
GACCACCTGG TACTGGTAAG AGTCATTTTG CCATCGGACT TGCTCTCTAT TACCCATCTG CTCGCATAGT
    17020      17030      17040      17050      17060      17070      17080

                 >< SfaNI
           >< SphI     >< PvuII
           >< PaeI     >< Psp5I
           >< NspI     >< NspBII
           >< NspHI  >< Fnu4HI                    > < Tru9I
  >< Bst1107I       > < NlaIII>< BspWI                  >< SspI
 >< AccI    >< NlaIII   >< AluI   >< BbvI            > < MseI
GTATACGGCA TGCTCTCATG CAGCTGTTGA TGCCCTATGT GAAAAGGCAT TAAAATATTT GCCCATAGAT
    17090      17100      17110      17120      17130      17140      17150
```

FIGURE 13.39

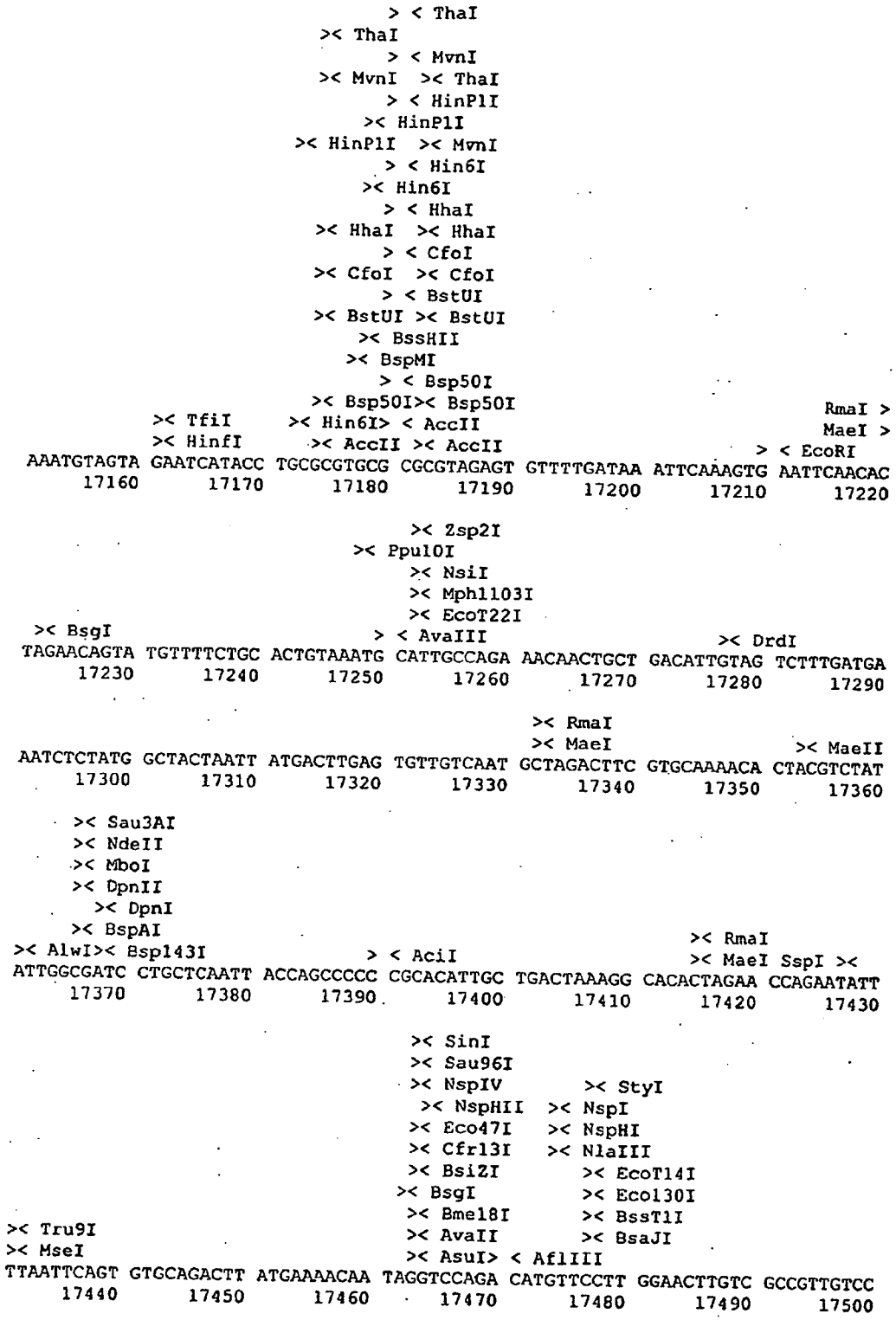

> < ThaI
>< ThaI
> < MvnI
>< MvnI >< ThaI
> < HinP1I
>< HinP1I
>< HinP1I >< MvnI
> < Hin6I
>< Hin6I
> < HhaI
>< HhaI >< HhaI
> < CfoI
>< CfoI >< CfoI
> < BstUI
>< BstUI >< BstUI
>< BssHII
>< BspMI
> < Bsp50I
>< Bsp50I>< Bsp50I
>< TfiI >< Hin6I> < AccII RmaI >
>< HinfI >< AccII >< AccII MaeI >
> < EcoRI
AAATGTAGTA GAATCATACC TGCGCGTGCG CGCGTAGAGT GTTTTGATAA ATTCAAAGTG AATTCAACAC
17160 17170 17180 17190 17200 17210 17220
>< Zsp2I
>< Ppu10I
>< NsiI
>< Mph1103I
>< EcoT22I
>< BsgI > < AvaIII >< DrdI
TAGAACAGTA TGTTTTCTGC ACTGTAAATG CATTGCCAGA AACAACTGCT GACATTGTAG TCTTTGATGA
17230 17240 17250 17260 17270 17280 17290
>< RmaI
>< MaeI >< MaeII
AATCTCTATG GCTACTAATT ATGACTTGAG TGTTGTCAAT GCTAGACTTC GTGCAAAACA CTACGTCTAT
17300 17310 17320 17330 17340 17350 17360
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< BspAI
>< AlwI>< Bsp143I > < AciI >< RmaI
>< MaeI SspI ><
ATTGGCGATC CTGCTCAATT ACCAGCCCCC CGCACATTGC TGACTAAAGG CACACTAGAA CCAGAATATT
17370 17380 17390 17400 17410 17420 17430
>< SinI
>< Sau96I
>< NspIV >< StyI
>< NspHII >< NspI
>< Eco47I >< NspHI
>< Cfr13I >< NlaIII
>< BsiZI >< EcoT14I
>< BsgI >< Eco130I
>< Bme18I >< BssT1I
>< Tru9I >< AvaII >< BsaJI
>< MseI >< AsuI> < AflIII
TTAATTCAGT GTGCAGACTT ATGAAAACAA TAGGTCCAGA CATGTTCCTT GGAACTTGTC GCCGTTGTCC
17440 17450 17460 17470 17480 17490 17500

FIGURE 13. 40

```
            >< HindII
            >< HincII                                    >< AluI
TGCTGAAATT GTTGACACTG TGAGTGCTTT AGTTTATGAC AATAAGCTAA AAGCACACAA GGATAAGTCA
     17510      17520      17530      17540      17550      17560      17570

><AluI                                          >< NlaIII
GCTCAATGCT TCAAAATGTT CTACAAAGGT GTTATTACAC ATGATGTTTC ATCTGCAATC AACAGACCTC
     17580      17590      17600      17610      17620      17630      17640

     >< MnlI
><EcoNI
  >< BslI                                                    >< HphI
  >< BsiYI                                                >< AluI
AAATAGGCGT TGTAAGAGAA TTTCTTACAC GCAATCCTGC TTGGAGAAAA GCTGTTTTTA TCTCACCTTA
     17650      17660      17670      17680      17690      17700      17710

            >< SfcI          >< DdeI                    >< TfiI
                 > < AluI    >< BfrI                    >< HinfI
TAATTCACAG AACGCTGTAG CTTCAAAAAT CTTAGGATTG CCTACGCAGA CTGTTGATTC ATCACAGGGT
     17720      17730      17740      17750      17760      17770      17780

                                                          > < HindII
          >< Tth111I                                      > < HincII
         >< AspI                                             >< AciI
TCTGAATATG ACTATGTCAT ATTCACACAA ACTACTGAAA CAGCACACTC TTGTAATGTC AACCGCTTCA
     17790      17800      17810      17820      17830      17840      17850

                                                  >< XhoII
                                                  >< Sau3AI
                                                  >< NdeII
                                                  >< MflI
                                                  >< MboI
                                                 >< MamI
                                                  >< DpnII
                                                    >< DpnI
                                                  >< BstYI
                                                  >< BspAI
                                                    >< Bsp143I
                                                 >< BsiBI
                                                 >< BsaBI
                                  >< BspWI        >< BglII
ATGTGGCTAT CACAAGGGCA AAAATTGGCA TTTTGTGCAT AATGTCTGAT AGAGATCTTT ATGACAAACT
     17860      17870      17880      17890      17900      17910      17920

          >< XbaI
           >< RmaI                                          >< MaeIII
           >< MaeI     >< MaeII                                BsrI ><
GCAATTTACA AGTCTAGAAA TACCACGTCG CAATGTGGCT ACATTACAAG CAGAAAATGT AACTGGACTT
     17930      17940      17950      17960      17970      17980      17990

            >< Sau3AI
            >< NdeII
                 >< MboII
            >< MboI
               > < FokI
            >< DpnII                          >< NlaIV
              >< DpnI                       >< Eco64I
            >< BspAI                          >< BscBI
><Tru9I       >< Bsp143I                    >< BanI          MnlI ><
><MseI>< SfcI      >< BbsI > < BsrI         >< AccB1I     >< DdeI
```

FIGURE 13.41

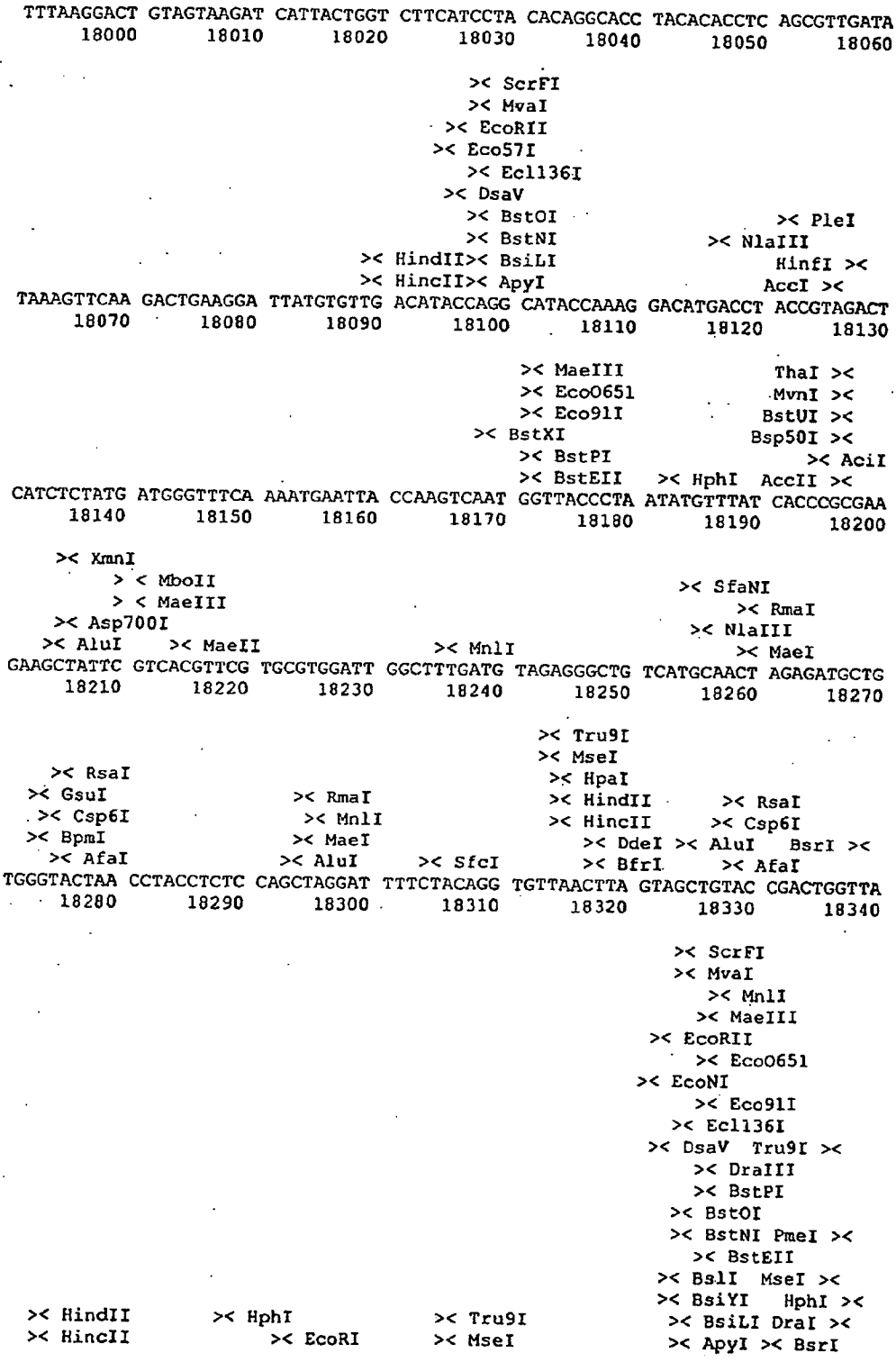

TTTAAGGACT GTAGTAAGAT CATTACTGGT CTTCATCCTA CACAGGCACC TACACACCTC AGCGTTGATA
18000 18010 18020 18030 18040 18050 18060
>< ScrFI
>< MvaI
>< EcoRII
>< Eco57I
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< PleI
>< NlaIII
>< HindII>< BsiLI
>< HincII>< ApyI
HinfI ><
AccI ><
TAAAGTTCAA GACTGAAGGA TTATGTGTTG ACATACCAGG CATACCAAAG GACATGACCT ACCGTAGACT
18070 18080 18090 18100 18110 18120 18130
>< MaeIII
>< Eco0651
>< Eco91I
>< BstXI
>< BstPI
>< BstEII
ThaI ><
MvnI ><
BstUI ><
Bsp50I ><
>< AciI
>< HphI AccII ><
CATCTCTATG ATGGGTTTCA AAATGAATTA CCAAGTCAAT GGTTACCCTA ATATGTTTAT CACCCGCGAA
18140 18150 18160 18170 18180 18190 18200
>< XmnI
>< MboII
>< MaeIII
>< Asp700I
>< AluI >< MaeII
>< MnlI
>< SfaNI
>< RmaI
>< NlaIII
>< MaeI
GAAGCTATTC GTCACGTTCG TGCGTGGATT GGCTTTGATG TAGAGGGCTG TCATGCAACT AGAGATGCTG
18210 18220 18230 18240 18250 18260 18270
>< Tru9I
>< MseI
>< HpaI
>< HindII
>< HincII
>< RsaI
>< GsuI
>< Csp6I
>< BpmI
>< AfaI
>< RmaI
>< MnlI
>< MaeI
>< AluI
>< SfcI
>< DdeI >< AluI
>< BfrI
>< RsaI
>< Csp6I
BsrI ><
>< AfaI
TGGGTACTAA CCTACCTCTC CAGCTAGGAT TTTCTACAGG TGTTAACTTA GTAGCTGTAC CGACTGGTTA
18280 18290 18300 18310 18320 18330 18340
>< ScrFI
>< MvaI
>< MnlI
>< MaeIII
>< EcoRII
>< Eco0651
>< EcoNI
>< Eco91I
>< Ecl136I
>< DsaV Tru9I ><
>< DraIII
>< BstPI
>< BstOI
>< BstNI PmeI ><
>< BstEII
>< BslI MseI ><
>< BsiYI HphI ><
>< BsiLI DraI ><
>< ApyI >< BsrI
>< HindII
>< HincII
>< HphI
>< EcoRI
>< Tru9I
>< MseI

FIGURE 13.42

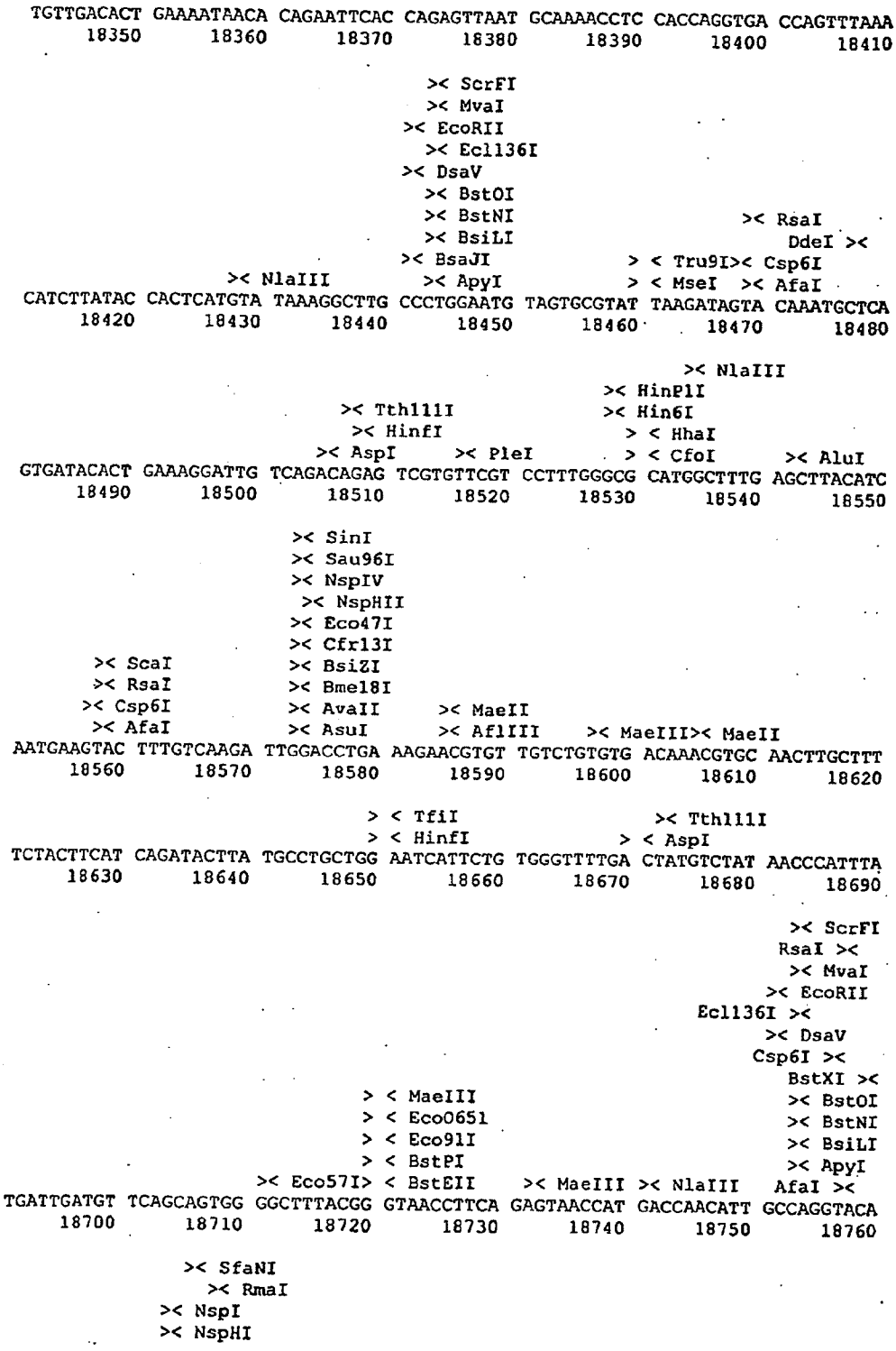

TGTTGACACT GAAAATAACA CAGAATTCAC CAGAGTTAAT GCAAAACCTC CACCAGGTGA CCAGTTTAAA
18350 18360 18370 18380 18390 18400 18410
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< BsaJI
>< RsaI
DdeI ><
> < Tru9I>< Csp6I
> < MseI >< AfaI
>< NlaIII
>< ApyI
CATCTTATAC CACTCATGTA TAAAGGCTTG CCCTGGAATG TAGTGCGTAT TAAGATAGTA CAAATGCTCA
18420 18430 18440 18450 18460 18470 18480
>< NlaIII
>< HinPlI
>< Hin6I
>< Tth111I
>< HinfI
> < HhaI
>< AspI
>< PleI
> < CfoI
>< AluI
GTGATACACT GAAAGGATTG TCAGACAGAG TCGTGTTCGT CCTTTGGGCG CATGGCTTTG AGCTTACATC
18490 18500 18510 18520 18530 18540 18550
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< Eco47I
>< Cfr13I
>< ScaI
>< BsiZI
>< RsaI
>< Bme18I
>< Csp6I
>< AvaII
>< MaeII
>< AfaI
>< AsuI
>< AflIII
>< MaeIII>< MaeII
AATGAAGTAC TTTGTCAAGA TTGGACCTGA AAGAACGTGT TGTCTGTGTG ACAAACGTGC AACTTGCTTT
18560 18570 18580 18590 18600 18610 18620
> < TfiI
> < HinfI
>< Tth111I
> < AspI
TCTACTTCAT CAGATACTTA TGCCTGCTGG AATCATTCTG TGGGTTTTGA CTATGTCTAT AACCCATTTA
18630 18640 18650 18660 18670 18680 18690
>< ScrFI
RsaI ><
>< MvaI
>< EcoRII
Ecl136I ><
>< DsaV
Csp6I ><
BstXI ><
> < MaeIII
> < Eco0651
> < Eco91I
> < BstPI
>< BstOI
>< BstNI
>< BsiLI
>< ApyI
>< Eco57I> < BstEII
>< MaeIII >< NlaIII
AfaI ><
TGATTGATGT TCAGCAGTGG GGCTTTACGG GTAACCTTCA GAGTAACCAT GACCAACATT GCCAGGTACA
18700 18710 18720 18730 18740 18750 18760
>< SfaNI
>< RmaI
>< NspI
>< NspHI

FIGURE 13.43

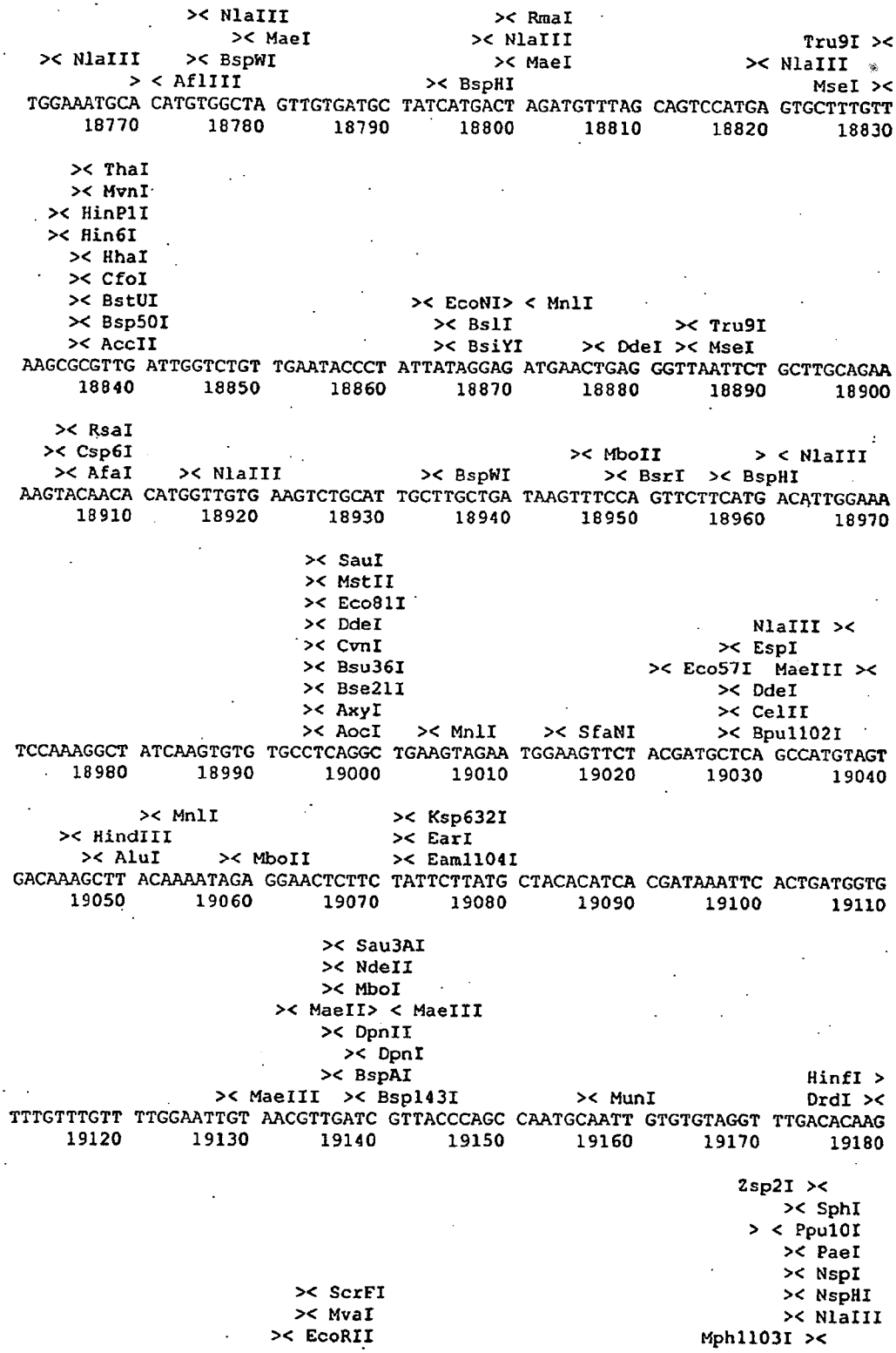

>< NlaIII
>< MaeI
>< NlaIII
>< BspWI
> < AflIII
TGGAAATGCA CATGTGGCTA GTTGTGATGC
18770 18780 18790
>< RmaI
>< NlaIII
>< MaeI
>< BspHI
TATCATGACT AGATGTTTAG
18800 18810
Tru9I ><
>< NlaIII
MseI ><
CAGTCCATGA GTGCTTTGTT
18820 18830
>< ThaI
>< MvnI
>< HinP1I
>< Hin6I
>< HhaI
>< CfoI
>< BstUI
>< Bsp50I
>< AccII
>< EcoNI> < MnlI
>< BslI
>< BsiYI
>< DdeI
>< Tru9I
>< MseI
AAGCGCGTTG ATTGGTCTGT TGAATACCCT ATTATAGGAG ATGAACTGAG GGTTAATTCT GCTTGCAGAA
18840 18850 18860 18870 18880 18890 18900
>< RsaI
>< Csp6I
>< AfaI
>< NlaIII
>< BspWI
>< MboII
>< BsrI
>< BspHI
> < NlaIII
AAGTACAACA CATGGTTGTG AAGTCTGCAT TGCTTGCTGA TAAGTTTCCA GTTCTTCATG ACATTGGAAA
18910 18920 18930 18940 18950 18960 18970
>< SauI
>< MstII
>< Eco81I
>< DdeI
>< CvnI
>< Bsu36I
>< Bse21I
>< AxyI
>< AocI
>< MnlI
>< SfaNI
NlaIII ><
>< EspI
>< Eco57I
MaeIII ><
>< DdeI
>< CelII
>< Bpu1102I
TCCAAAGGCT ATCAAGTGTG TGCCTCAGGC TGAAGTAGAA TGGAAGTTCT ACGATGCTCA GCCATGTAGT
18980 18990 19000 19010 19020 19030 19040
>< MnlI
>< HindIII
>< AluI
>< MboII
>< Ksp632I
>< EarI
>< Eam1104I
GACAAAGCTT ACAAAATAGA GGAACTCTTC TATTCTTATG CTACACATCA CGATAAATTC ACTGATGGTG
19050 19060 19070 19080 19090 19100 19110
>< Sau3AI
>< NdeII
>< MboI
>< MaeII> < MaeIII
>< DpnII
>< DpnI
>< BspAI
>< MaeIII
>< Bsp143I
>< MunI
HinfI >
DrdI ><
TTTGTTTGTT TTGGAATTGT AACGTTGATC GTTACCCAGC CAATGCAATT GTGTGTAGGT TTGACACAAG
19120 19130 19140 19150 19160 19170 19180
Zsp2I ><
>< SphI
> < Ppu10I
>< PaeI
>< NspI
>< NspHI
>< NlaIII
Mph1103I ><
>< ScrFI
>< MvaI
>< EcoRII

FIGURE 13A4

```
                          >< Ecl136I                                  >< GsuI
                        >< DsaV                                       EcoT22I ><
                          >< BstOI                                        >< BsmI
                          >< BstNI                                     >< BscCI
                          >< BsiLI                                    >< BpmI >< NsiI
        >< PleI           >< ApyI                                        >< AvaIII
AGTCTTGTCA AACTTGAACT TACCAGGCTG TGATGGTGGT AGTTTGTATG TGAATAAGCA TGCATTCCAC
     19190      19200      19210      19220      19230      19240      19250

                                    >< Tru9I
                                       > < MunI
          >< TthHB8I                >< MseI
><BcgI/a  >< TaqI                    >< DraI
     >< AluI                         >< BcgI
ACTCCAGCTT TCGATAAAAG TGCATTTACT AATTTAAAGC AATTGCCTTT CTTTTACTAT TCTGATAGTC
     19260      19270      19280      19290      19300      19310      19320

              >< PleI                                                SfaNI ><
               >< NlaIII                                               >< MaeII
             >< BsmAI                                               BsaAI ><
     >< HinfI>< Alw26I                                             AflIII ><
CTTGTGAGTC TCATGGCAAA CAAGTAGTGT CGGATATTGA TTATGTTCCA CTCAAATCTG CTACGTGTAT
     19330      19340      19350      19360      19370      19380      19390

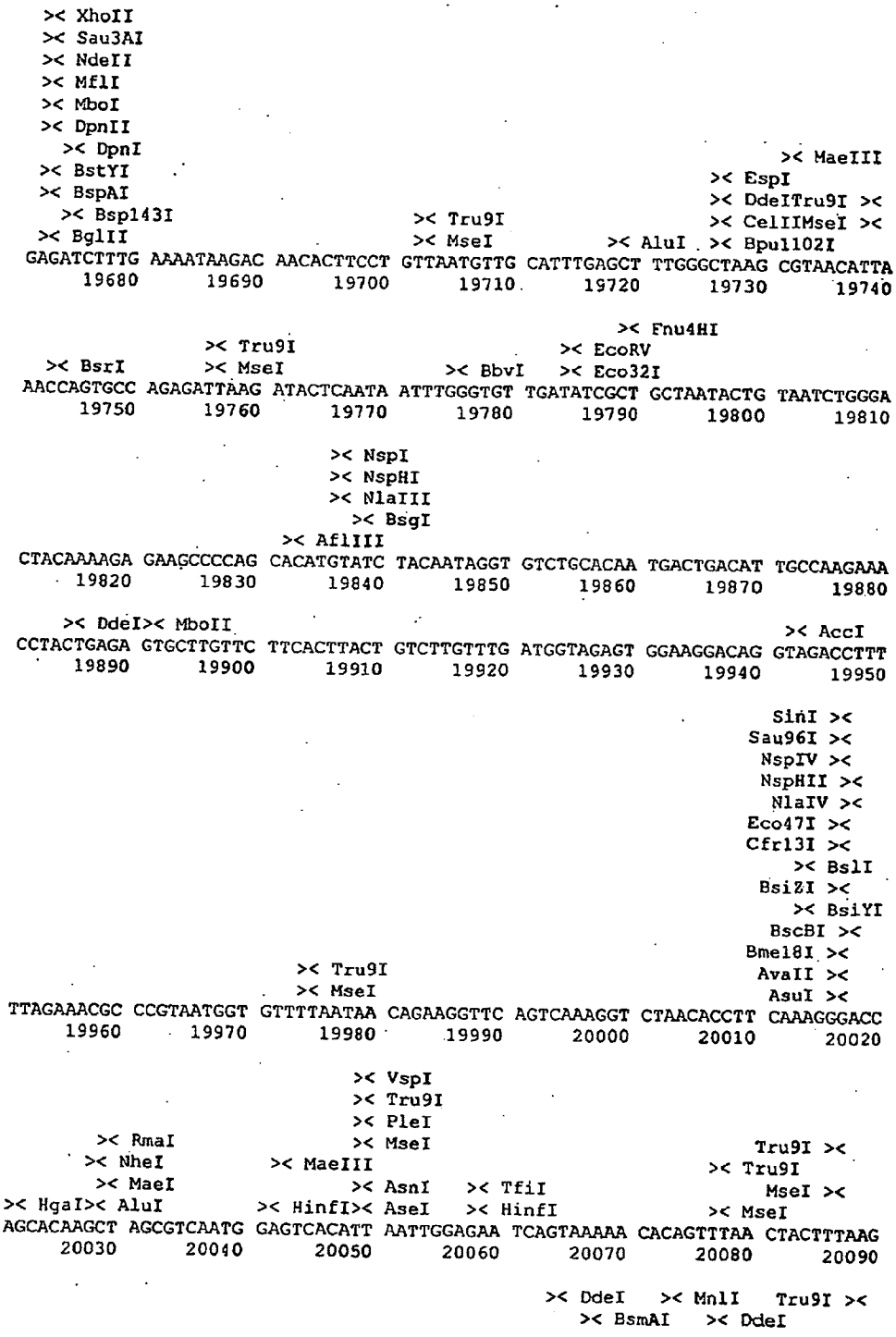
                                                                     Zsp2I >
                                                                  >< ScaI
                                                                   Ppu10I ><
                                                                  >< RsaINsiI >
                                                                     Mph1103I >
                                                                >< SfaNIEcoT22I >
                                                          > < RsaI >< Csp6I
                                                          >< Csp6I      AvaIII ><
                                                    >< NlaIII> < AfaI  >< AfaI
TACACGATGC AATTTAGGTG GTGCTGTTTG CAGACACCAT GCAAATGAGT ACCGACAGTA CTTGGATGCA
     19400      19410      19420      19430      19440      19450      19460

     >< FokI
TATAATATGA TGATTTCTGC TGGATTTAGC CTATGGATTT ACAAACAATT TGATACTTAT AACCTGTGGA
     19470      19480      19490      19500      19510      19520      19530

          >< ScrFI
          >< HvaI
            >< MaeIII
        >< EcoRII
          >< Ecl136I
        >< DsaV
          >< BstOI
          >< BstNI
          >< BsiLI                                  >< Tru9I
          >< ApyI                                   >< MseI
ATACATTTAC CAGGTTACAG AGTTTAGAAA ATGTGGCTTA TAATGTTGTT AATAAAGGAC ACTTTGATGG
     19540      19550      19560      19570      19580      19590      19600

   >< SgrAI
     >< NaeI
    >< MspI                  > < VspI
    >< HpaII                 > < Tru9I
    >< HapII                 > < MseI
   >< Cfr10I                 > < AsnI
         >< BspWI            > < AseI
ACACGCCGGC GAAGCACCTG TTTCCATCAT TAATAATGCT GTTTACACAA AGGTAGATGG TATTGATGTG
     19610      19620      19630      19640      19650      19660      19670
```

FIGURE 13. 45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>< NdeII
>< MflI
>< MboI
>< DpnII
>< DpnI
>< BstYI
>< BspAI
>< Bsp143I
>< BglII
>< Tru9I
>< MseI
>< AluI
>< MaeIII
>< EspI
>< DdeITru9I ><
>< CelIIMseI ><
>< Bpu1102I
GAGATCTTTG AAAATAAGAC AACACTTCCT GTTAATGTTG CATTTGAGCT TTGGGCTAAG CGTAACATTA
19680 19690 19700 19710 19720 19730 19740
>< Fnu4HI
>< Tru9I
>< EcoRV
>< BsrI
>< MseI
>< BbvI
>< Eco32I
AACCAGTGCC AGAGATTAAG ATACTCAATA ATTTGGGTGT TGATATCGCT GCTAATACTG TAATCTGGGA
19750 19760 19770 19780 19790 19800 19810
>< NspI
>< NspHI
>< NlaIII
>< BsgI
>< AflIII
CTACAAAAGA GAAGCCCCAG CACATGTATC TACAATAGGT GTCTGCACAA TGACTGACAT TGCCAAGAAA
19820 19830 19840 19850 19860 19870 19880
>< DdeI>< MboII
>< AccI
CCTACTGAGA GTGCTTGTTC TTCACTTACT GTCTTGTTTG ATGGTAGAGT GGAAGGACAG GTAGACCTTT
19890 19900 19910 19920 19930 19940 19950
SinI ><
Sau96I ><
NspIV ><
NspHII ><
NlaIV ><
Eco47I ><
Cfr13I ><
>< BslI
BsiZI ><
>< BsiYI
BscBI ><
Bme18I ><
AvaII ><
AsuI ><
>< Tru9I
>< MseI
TTAGAAACGC CCGTAATGGT GTTTTAATAA CAGAAGGTTC AGTCAAAGGT CTAACACCTT CAAAGGGACC
19960 19970 19980 19990 20000 20010 20020
>< VspI
>< Tru9I
>< PleI
>< RmaI
>< MseI
>< NheI
>< MaeIII
Tru9I ><
>< Tru9I
>< MaeI
>< AsnI
>< TfiI
MseI ><
>< HgaI>< AluI
>< HinfI>< AseI
>< HinfI
>< MseI
AGCACAAGCT AGCGTCAATG GAGTCACATT AATTGGAGAA TCAGTAAAAA CACAGTTTAA CTACTTTAAG
20030 20040 20050 20060 20070 20080 20090
>< DdeI
>< MnlI
Tru9I ><
>< BsmAI
>< DdeI

FIGURE 1346

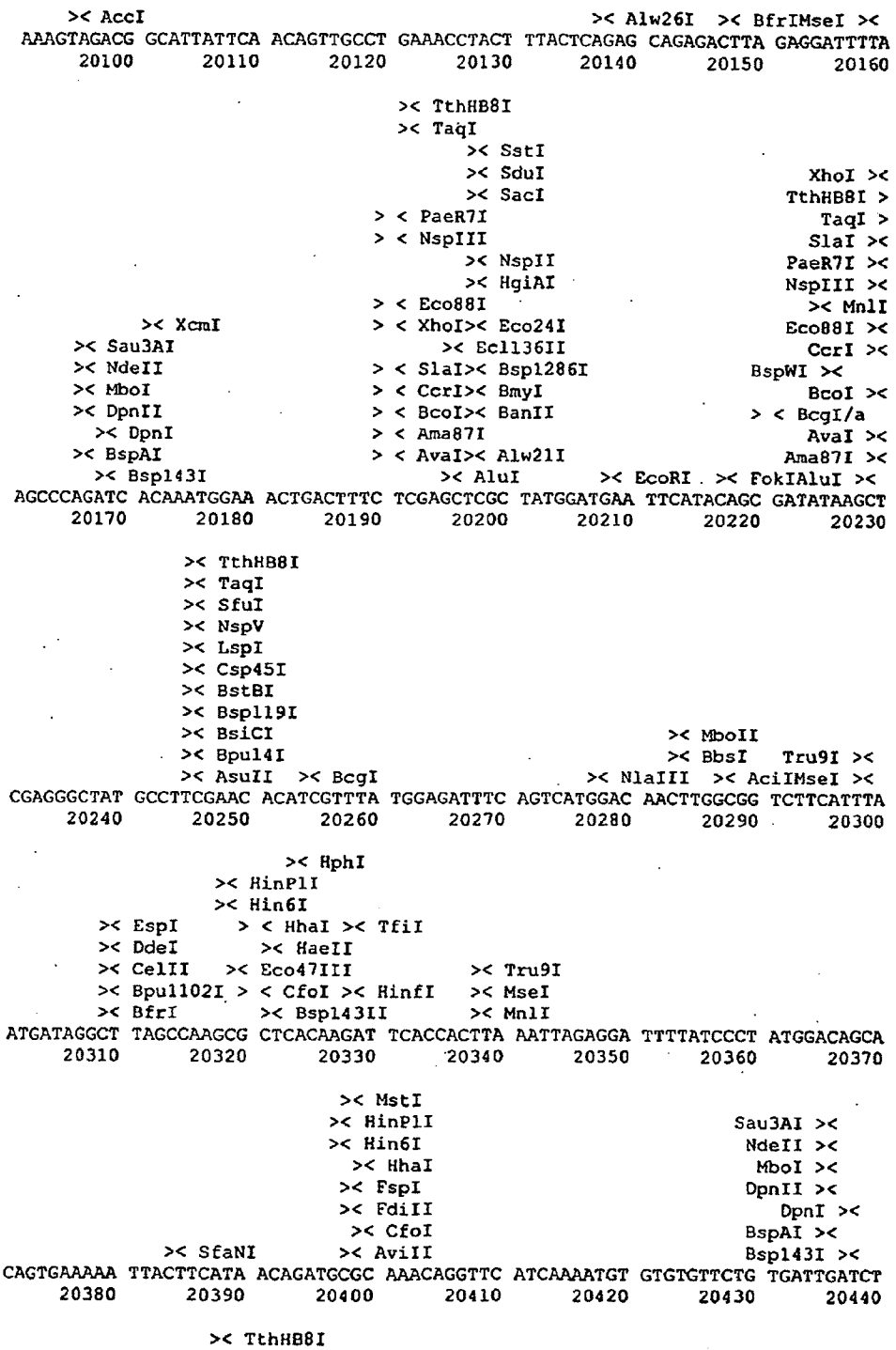

>< AccI
>< Alw26I >< BfrIMseI ><
AAAGTAGACG GCATTATTCA ACAGTTGCCT GAAACCTACT TTACTCAGAG CAGAGACTTA GAGGATTTTA
20100 20110 20120 20130 20140 20150 20160
>< TthHB8I
>< TaqI
>< SstI
>< SduI
>< SacI
> < PaeR7I
> < NspIII
>< NspII
>< HgiAI
> < Eco88I
> < XhoI>< Eco24I
>< Ecl136II
> < SlaI>< Bsp1286I
> < CcrI>< BmyI
> < BcoI>< BanII
> < Ama87I
> < AvaI>< Alw21I
>< AluI
XhoI ><
TthHB8I >
TaqI >
SlaI ><
PaeR7I ><
NspIII ><
>< MnlI
Eco88I ><
CcrI ><
BspWI ><
BcoI ><
> < BcgI/a
AvaI ><
Ama87I ><
>< XcmI
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< BspAI
>< Bsp143I
>< EcoRI >< FokIAluI ><
AGCCCAGATC ACAAATGGAA ACTGACTTTC TCGAGCTCGC TATGGATGAA TTCATACAGC GATATAAGCT
20170 20180 20190 20200 20210 20220 20230
>< TthHB8I
>< TaqI
>< SfuI
>< NspV
>< LspI
>< Csp45I
>< BstBI
>< Bspl19I
>< BsiCI
>< Bpul4I
>< AsuII >< BcgI
>< MboII
>< BbsI Tru9I ><
>< NlaIII >< AciIMseI ><
CGAGGGCTAT GCCTTCGAAC ACATCGTTTA TGGAGATTTC AGTCATGGAC AACTTGGCGG TCTTCATTTA
20240 20250 20260 20270 20280 20290 20300
>< HphI
>< HinPlI
>< Hin6I
>< EspI
>< DdeI
>< CelII
>< Bpul102I
>< BfrI
> < HhaI >< TfiI
>< HaeII
>< Eco47III
> < CfoI >< HinfI
>< Bsp143II
>< Tru9I
>< MseI
>< MnlI
ATGATAGGCT TAGCCAAGCG CTCACAAGAT TCACCACTTA AATTAGAGGA TTTTATCCCT ATGGACAGCA
20310 20320 20330 20340 20350 20360 20370
>< MstI
>< HinPlI
>< Hin6I
>< HhaI
>< FspI
>< FdiII
>< CfoI
>< SfaNI
>< AviII
Sau3AI ><
NdeII ><
MboI ><
DpnII ><
DpnI ><
BspAI ><
Bsp143I ><
CAGTGAAAAA TTACTTCATA ACAGATGCGC AAACAGGTTC ATCAAAATGT GTGTGTTCTG TGATTGATCT
20380 20390 20400 20410 20420 20430 20440
>< TthHB8I

FIGURE 13.47

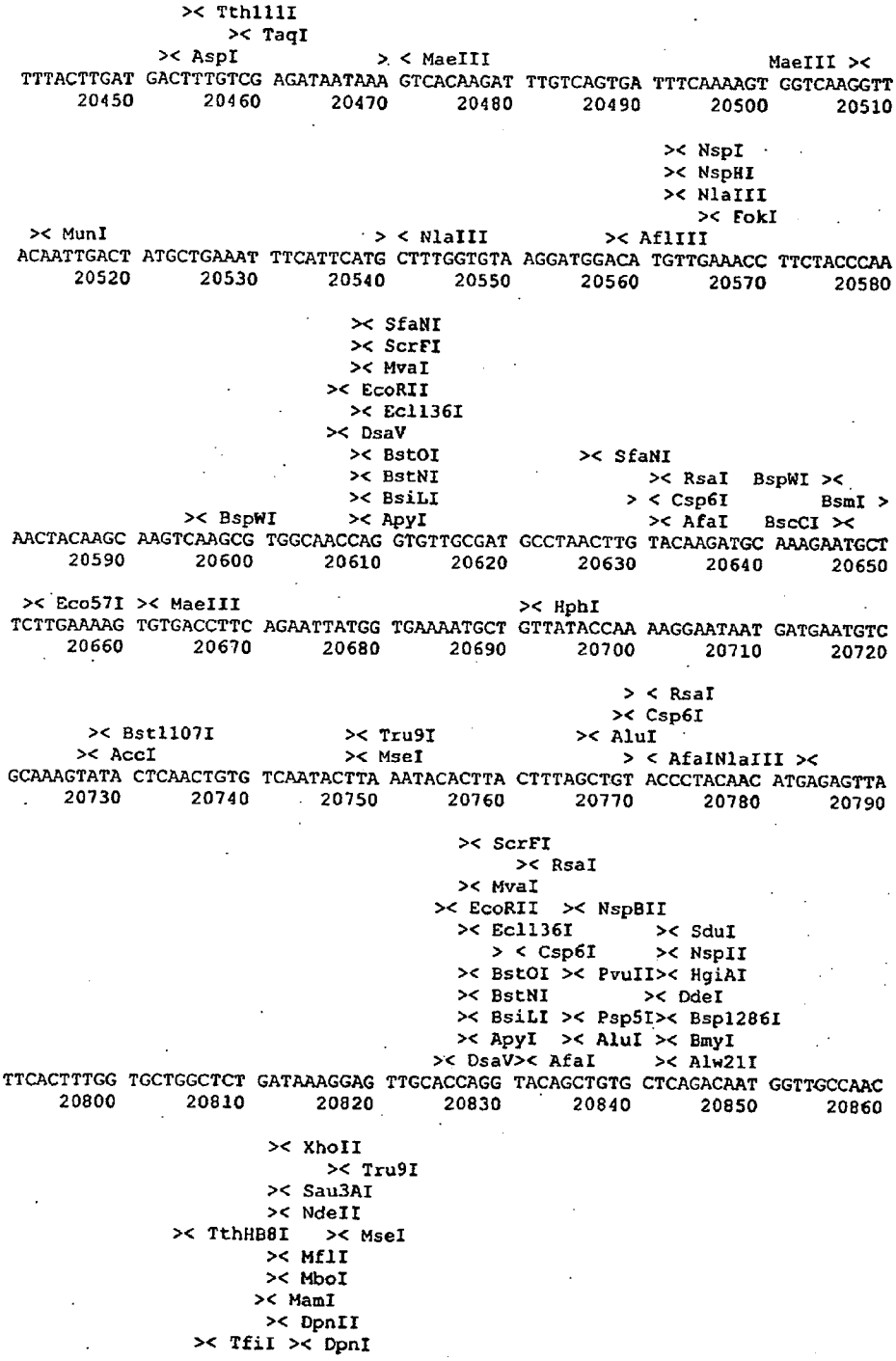

>< Tth111I
>< TaqI
>< AspI
> < MaeIII
MaeIII ><
TTTACTTGAT GACTTTGTCG AGATAATAAA GTCACAAGAT TTGTCAGTGA TTTCAAAAGT GGTCAAGGTT
20450 20460 20470 20480 20490 20500 20510
>< NspI
>< NspHI
>< NlaIII
>< FokI
>< MunI
> < NlaIII
>< AflIII
ACAATTGACT ATGCTGAAAT TTCATTCATG CTTTGGTGTA AGGATGGACA TGTTGAAACC TTCTACCCAA
20520 20530 20540 20550 20560 20570 20580
>< SfaNI
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< SfaNI
>< BstNI
>< RsaI BspWI ><
>< BsiLI
> < Csp6I BsmI >
>< BspWI
>< ApyI
>< AfaI BscCI ><
AACTACAAGC AAGTCAAGCG TGGCAACCAG GTGTTGCGAT GCCTAACTTG TACAAGATGC AAAGAATGCT
20590 20600 20610 20620 20630 20640 20650
>< Eco57I >< MaeIII
>< HphI
TCTTGAAAAG TGTGACCTTC AGAATTATGG TGAAAATGCT GTTATACCAA AAGGAATAAT GATGAATGTC
20660 20670 20680 20690 20700 20710 20720
> < RsaI
>< Csp6I
>< Bst1107I
>< Tru9I
>< AluI
>< AccI
>< MseI
> < AfaINlaIII ><
GCAAAGTATA CTCAACTGTG TCAATACTTA AATACACTTA CTTTAGCTGT ACCCTACAAC ATGAGAGTTA
20730 20740 20750 20760 20770 20780 20790
>< ScrFI
>< RsaI
>< MvaI
>< EcoRII >< NspBII
>< Ecl136I >< SduI
> < Csp6I >< NspII
>< BstOI >< PvuII>< HgiAI
>< BstNI >< DdeI
>< BsiLI >< Psp5I>< Bsp1286I
>< ApyI >< AluI >< BmyI
>< DsaV>< AfaI >< Alw21I
TTCACTTTGG TGCTGGCTCT GATAAAGGAG TTGCACCAGG TACAGCTGTG CTCAGACAAT GGTTGCCAAC
20800 20810 20820 20830 20840 20850 20860
>< XhoII
>< Tru9I
>< Sau3AI
>< NdeII
>< TthHB8I >< MseI
>< MflI
>< MboI
>< MamI
>< DpnII
>< TfiI >< DpnI

FIGURE 13. 48

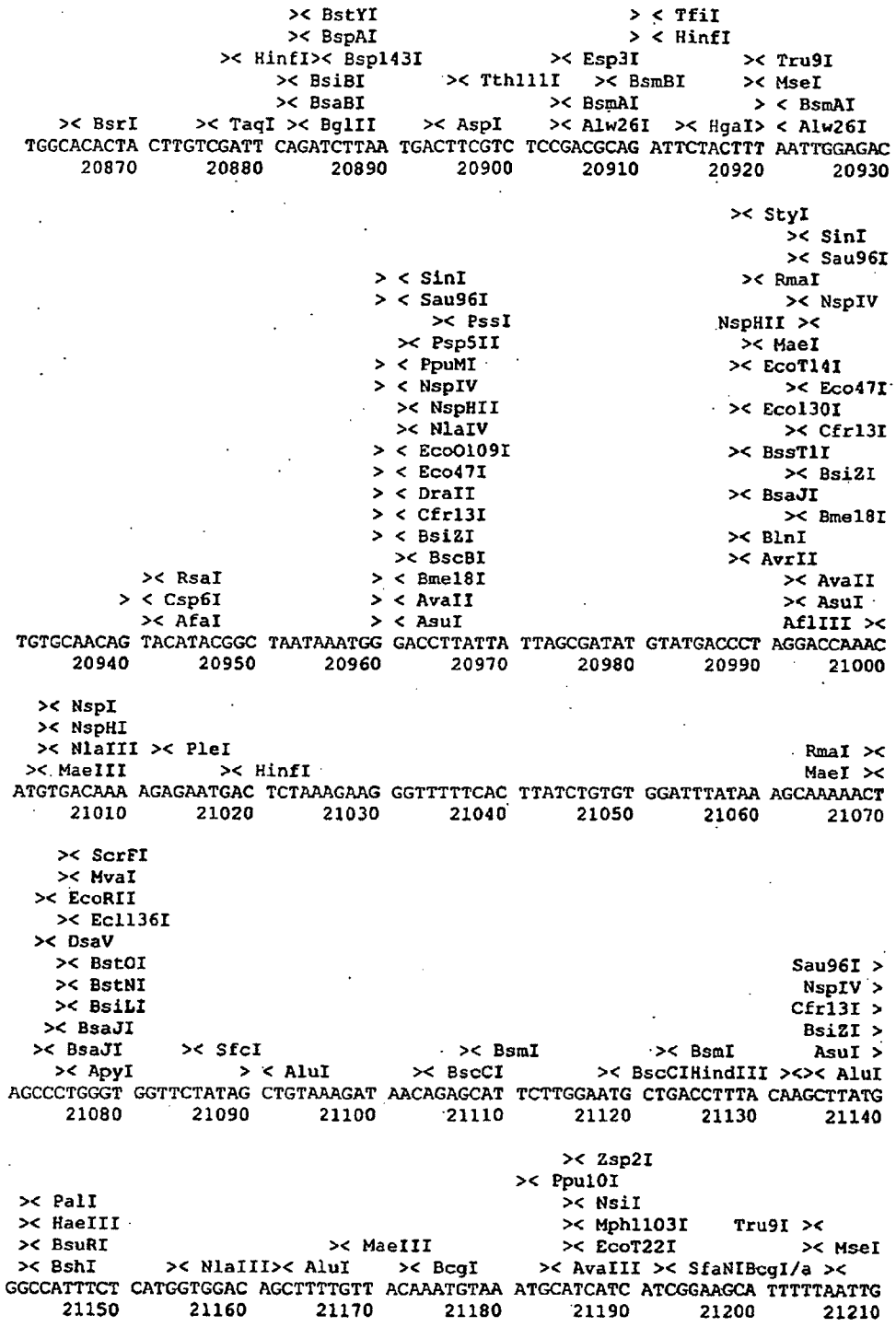

>< BstYI
>< BspAI
>< HinfI>< Bsp143I
>< BsiBI
>< BsaBI
> < TfiI
> < HinfI
>< Esp3I
>< BsmBI
>< BsmAI
>< Tru9I
>< MseI
> < BsmAI
>< BsrI
>< TaqI >< BglII
>< AspI
>< Alw26I
>< HgaI> < Alw26I
TGGCACACTA CTTGTCGATT CAGATCTTAA TGACTTCGTC TCCGACGCAG ATTCTACTTT AATTGGAGAC
20870 20880 20890 20900 20910 20920 20930
>< StyI
>< SinI
>< Sau96I
> < SinI
> < Sau96I
>< PssI
>< Psp5II
> < PpuMI
> < NspIV
>< NspHII
>< NlaIV
> < Eco0109I
> < Eco47I
> < DraII
> < Cfr13I
> < BsiZI
>< BscBI
>< RmaI
>< NspIV
NspHII ><
>< MaeI
>< EcoT14I
>< Eco47I
>< Eco130I
>< Cfr13I
>< BssT1I
>< Bsi2I
>< BsaJI
>< Bme18I
>< BlnI
>< AvrII
>< RsaI
> < Bme18I
> < AvaII
> < AsuI
>< Csp6I
>< AfaI
>< AvaII
>< AsuI
AflIII ><
TGTGCAACAG TACATACGGC TAATAAATGG GACCTTATTA TTAGCGATAT GTATGACCCT AGGACCAAAC
20940 20950 20960 20970 20980 20990 21000
>< NspI
>< NspHI
>< NlaIII >< PleI
>< MaeIII
>< HinfI
RmaI ><
MaeI ><
ATGTGACAAA AGAGAATGAC TCTAAAGAAG GGTTTTTCAC TTATCTGTGT GGATTTATAA AGCAAAAACT
21010 21020 21030 21040 21050 21060 21070
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< BsaJI
>< BsaJI
>< SfcI
>< BsmI
>< BsmI
Sau96I >
NspIV >
Cfr13I >
BsiZI >
AsuI >
>< ApyI
> < AluI
>< BscCI
>< BscCIHindIII ><>< AluI
AGCCCTGGGT GGTTCTATAG CTGTAAAGAT AACAGAGCAT TCTTGGAATG CTGACCTTTA CAAGCTTATG
21080 21090 21100 21110 21120 21130 21140
>< Zsp2I
>< Ppu10I
>< PalI
>< HaeIII
>< BsuRI
>< BshI
>< NlaIII>< AluI
>< MaeIII
>< BcgI
>< NsiI
>< Mph1103I
>< EcoT22I
Tru9I ><
>< AvaIII >< SfaNIBcgI/a ><
>< MseI
GGCCATTTCT CATGGTGGAC AGCTTTTGTT ACAAATGTAA ATGCATCATC ATCGGAAGCA TTTTTAATTG
21150 21160 21170 21180 21190 21200 21210

FIGURE 13.49

```
                                                                    >< Zsp2I
                                                                      >< SphI
                                                                  >< Ppu10I
                                                                      >< PaeI
                                                                      >< NspI
                                                                      >< NspHI
                                                                    >< NsiI
                                                                      >< NlaIII
                                                                  > < NlaIII
                                                                    >< Mph1103I
                                                                    >< EcoT22I
                                                                  > <. AvaIII      >< MnlI
GGGCTAACTA TCTTGGCAAG CCGAAGGAAC AAATTGATGG CTATACCATG CATGCTAACT ACATTTTCTG
     21220      21230      21240      21250      21260      21270      21280

                                                                          Tru9I ><
                    >< MboII                                               >< Tru9I
                     >< GsuI                                               MseI ><
                     >< BsrI                                               >< MseI
                     >< BpmI                                                MnlI ><
                    >< BbsI                                  >< NlaIII     >< MnlI
GAGGAACACA AATCCTATCC AGTTGTCTTC CTATTCACTC TTTGACATGA GCAAATTTCC TCTTAAATTA
     21290      21300      21310      21320      21330      21340      21350

                         >< Tru9I
                         >< MseI
                        >< Esp4I> < TfiI
                         >< BsmAI                                Ksp632I ><
                         >< Alw26I                          >< MboII      >< EarI
                        >< AflII> < HinfI                      Eam1104I ><
AGAGGAACTG CTGTAATGTC TCTTAAGGAG AATCAAATCA ATGATATGAT TTATTCTCTT CTGGAAAAAG
     21360      21370      21380      21390      21400      21410      21420

                                                                    >< Tru9I
                                                                    >< MseI
                                                                     >< HindII
                                                                     >< HincII
                                                                     >< HpaI AflIII >
GTAGGCTTAT CATTAGAGAA AACAACAGAG TTGTGGTTTC AAGTGATATT CTTGTTAACA ACTAAACGAA
     21430      21440      21450      21460      21470      21480      21490

                                                                    >< VneI
                                                                    >< SnoI
                                                                        >< SduI
                                                                        >< NspII
                                                                  >< HpaII
                                                                        >< HgiAI
                                                                  >< HapII
                                                                 >< Cfr10I
                                                                        >< Bsp1286I
                                                                  >< MspI>< BmyI
  >< NspI                              >< SpeI                          >< ApaLI
  >< NspHI                              >< RmaI                         >< Alw44I
  >< NlaIII                             >< MaeI  >< MaeIII  >< AgeI >< Alw21I
CATGTTTATT TTCTTATTAT TTCTTACTCT CACTAGTGGT AGTGACCTTG ACCGGTGCAC CACTTTTGAT
     21500      21510      21520      21530      21540      21550      21560

         > < AluI                        >< MnlI
GATGTTCAAG CTCCTAATTA CACTCAACAT ACTTCATCTA TGAGGGGGGT TTACTATCCT GATGAAATTT
     21570      21580      21590      21600      21610      21620      21630

  >< Sau3AI
```

FIGURE 13. 50

```
   >< NdeII
   >< MboI
   >< DpnII
    >< DpnI          >< Tru9I
   >< BspAI          >< MseI  > < MboII
    >< Bspl43I           >< DdeI                          >< MaeIII
TTAGATCAGA CACTCTTTAT TTAACTCAGG ATTTATTTCT TCCATTTTAT TCTAATGTTA CAGGGTTTCA
    21640      21650      21660      21670      21680      21690      21700

    >< VspI
    >< Tru9I
    >< MseI
    >< AsnI                                >< Tru9I          >< FokI
    >< AseI  >< MaeII                      >< MseI >< BbvI       > < Fnu4HI
TACTATTAAT CATACGTTTG GCAACCCTGT CATACCTTTT AAGGATGGTA TTTATTTTGC TGCCACAGAG
    21710      21720      21730      21740      21750      21760      21770

                    >< BslI
               >< DsaI>< BsiYI              >< NlaIII
               >< BsaJI                          > < MaeIII
AAATCAAATG TTGTCCGTGG TTGGGTTTTT GGTTCTACCA TGAACAACAA GTCACAGTCG GTGATTATTA
    21780      21790      21800      21810      21820      21830      21840

                                  >< NspI
><Tru9I                           >< NspHI
><MseI                            >< NlaIII
 >< HphI                         >< MaeIII       >< MaeIII
TTAACAATTC TACTAATGTT GTTATACGAG CATGTAACTT TGAATTGTGT GACAACCCTT TCTTTGCTGT
    21850      21860      21870      21880      21890      21900      21910

       >< StyI                                    >< Zsp2I
            >< NlaIII                                >< Tru9I
       >< NcoI >< RsaI                          >< Ppu10I   TthHB8I ><
       >< EcoT14I                                  >< NsiI         >< TaqI
       >< Eco130I                                     >< MseI    SfaNI ><
       >< DsaI>< Csp6I                             >< Mph1103I     RsaI ><
       >< BssT1I                          >< TthHB8I >< EcoT22I    Csp6I ><
       >< BsaJI>< AfaI                    >< TaqI  >< AvaIII         AfaI ><
TTCTAAACCC ATGGGTACAC AGACACATAC TATGATATTC GATAATGCAT TTAATTGCAC TTTCGAGTAC
    21920      21930      21940      21950      21960      21970      21980

                                              >< Tru9I
                                              >< MseI
                                               >< DraI
ATATCTGATG CCTTTTCGCT TGATGTTTCA GAAAAGTCAG GTAATTTTAA ACACTTACGA GAGTTTGTGT
    21990      22000      22010      22020      22030      22040      22050

                                                                  >< Sau3AI
                                                                  >< NdeII
                                                                  >< MboI
                                                                  >< DpnII
><Tru9I                                                             >< DpnI
><MseI                                                            >< BspAI
 >< DraI                                          >< SfcI     Bspl43I ><
TTAAAAATAA AGATGGGTTT CTCTATGTTT ATAAGGGCTA TCAACCTATA GATGTAGTTC GTGATCTACC
    22060      22070      22080      22090      22100      22110      22120

                                              >< Tru9I
       >< Tru9I              > < Tru9I        >< MseI
       >< MseI               > < MseI         >< MnlI
TTCTGGTTTT AACACTTTGA AACCTATTTT TAAGTTGCCT CTTGGTATTA ACATTACAAA TTTTAGAGCC
    22130      22140      22150      22160      22170      22180      22190
```

FIGURE 13.51

```
                                                  > < SduI>< SfcI
                                                        >< PvuII
                                                        >< Psp5I
                                                  > < NspII
                                                        >< NspBII
                                                  > < MaeII  > < Fnu4HI
                                                  > < Bspl286I >< PstI       Tru9I >
                                 >< BspMI         > < BmyI>< Fnu4HI           MseI >
          >< HphI                              >< BbvI       >< AluI          >< BbvI
ATTCTTACAG CCTTTTCACC TGCTCAAGAC ATTTGGGGCA CGTCAGCTGC AGCCTATTTT GTTGGCTATT
     22200      22210      22220      22230      22240      22250      22260

                                                     >< SfaNI
                                                     >< RsaI
                                                  > < Csp6I
><DraI                                               >< AfaI     >< AlwNI
TAAAGCCAAC TACATTTATG CTCAAGTATG ATGAAAATGG TACAATCACA GATGCTGTTG ATTGTTCTCA
     22270      22280      22290      22300      22310      22320      22330

                                    > < Tru9I
                                    > < MseI
                                          >< AluI
AAATCCACTT GCTGAACTCA AATGCTCTGT TAAGAGCTTT GAGATTGACA AAGGAATTTA CCAGACCTCT
     22340      22350      22360      22370      22380      22390      22400

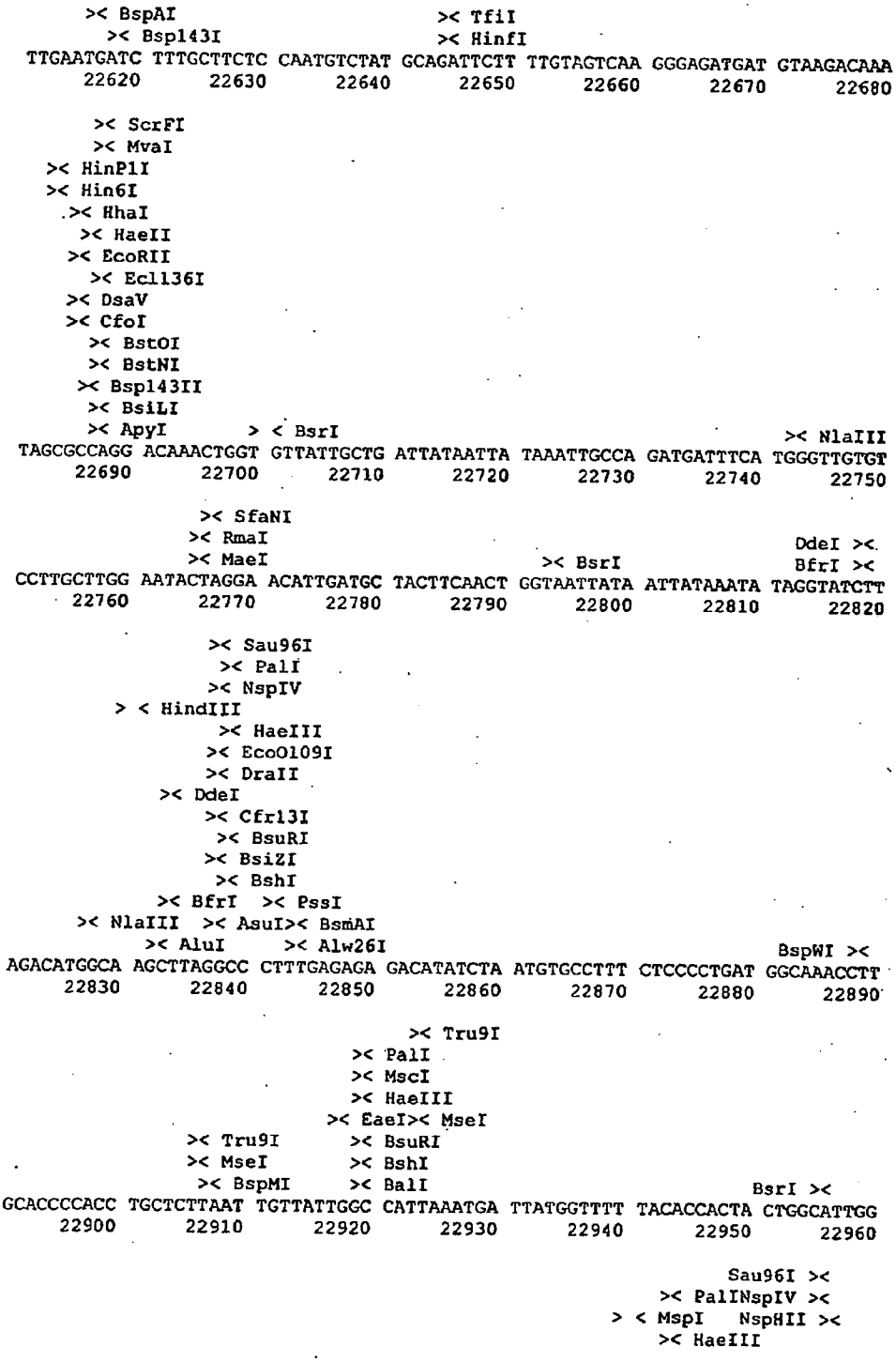
                     >< SauI
                     >< MstII
                     >< Eco81I
                     >< DdeI
                     >< CvnI
                     >< Bsu36I
                     >< Bse21I
                     >< AxyI                 >< TfiI
     >< MnlI         >< AocI   >< MnlI       >< HinfI   >< SspI              >< MnlI
AATTTCAGGG TTGTTCCCTC AGGAGATGTT GTGAGATTCC CTAATATTAC AAACTTGTGT CCTTTTGGAG
     22410      22420      22430      22440      22450      22460      22470

                                          >< Zsp2I
                                       >< Ppu10I
                                          >< NsiI
                                            > < NlaIII
                                          >< Mph1103I
     >< Tru9I                             >< EcoT22I
     >< MseI                           >< AvaIII
AGGTTTTTAA TGCTACTAAA TTCCCTTCTG TCTATGCATG GGAGAGAAAA AAAATTTCTA ATTGTGTTGC
     22480      22490      22500      22510      22520      22530      22540

                >< SduI
                >< NspII
                >< HgiAI
                >< Bspl286I
                >< BmyI                          >< Tru9I
                >< Alw21I                        >< MseI              DdeI ><
TGATTACTCT GTGCTCTACA ACTCAACATT TTTTTCAACC TTTAAGTGCT ATGGCGTTTC TGCCACTAAG
     22550      22560      22570      22580      22590      22600      22610

     >< Sau3AI
     >< NdeII
     >< MboI
     >< DpnII
       >< DpnI
```

FIGURE 1352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>< Bsp143I
>< TfiI
>< HinfI
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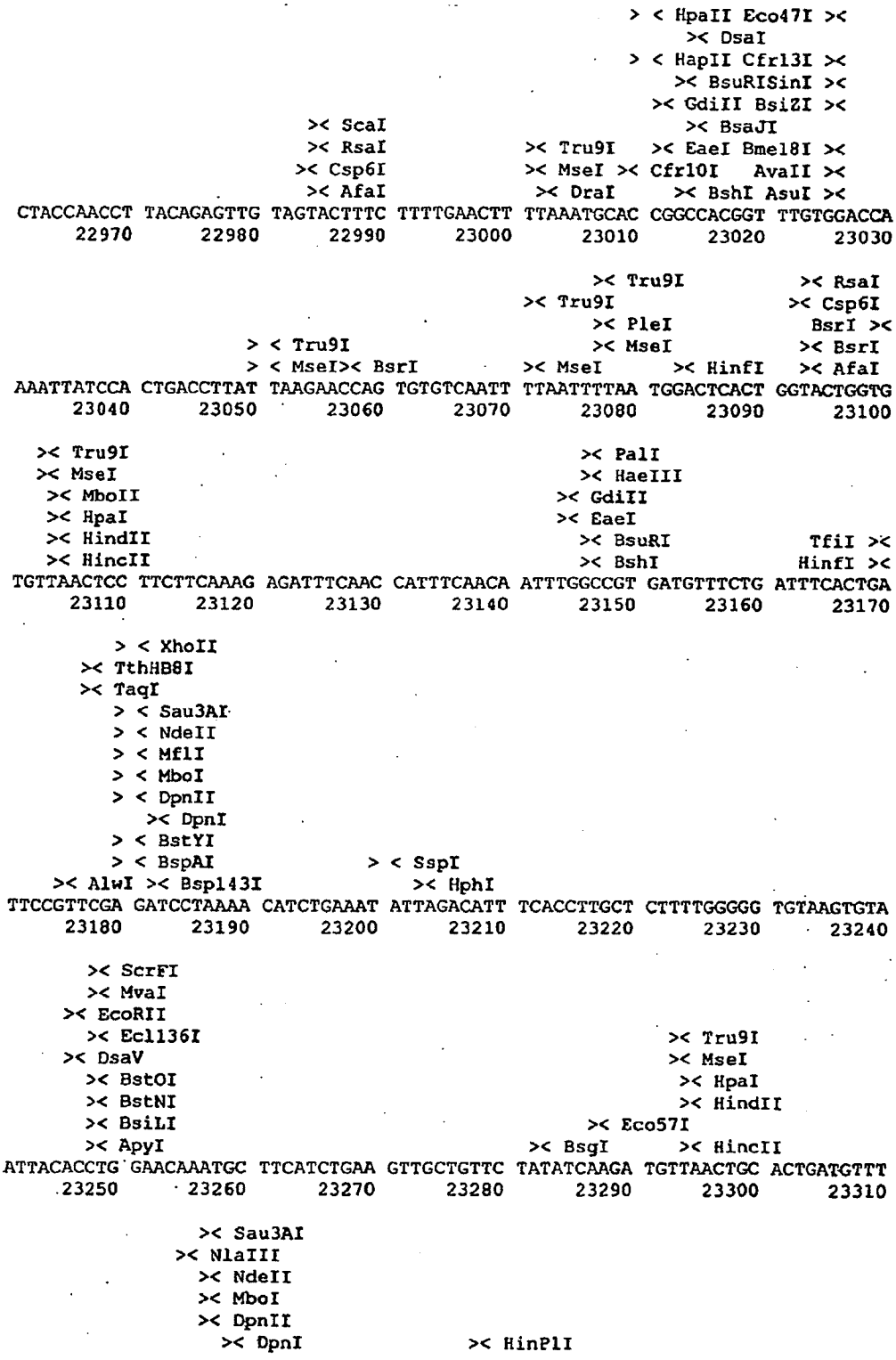
22620 22630 22640 22650 22660 22670 22680
>< ScrFI
>< MvaI
>< HinP1I
>< Hin6I
>< RhaI
>< HaeII
>< EcoRII
>< Ecl136I
>< DsaV
>< CfoI
>< BstOI
>< BstNI
>< Bsp143II
>< BsiLI
>< ApyI
> < BsrI
>< NlaIII
TAGCGCCAGG ACAAACTGGT GTTATTGCTG ATTATAATTA TAAATTGCCA GATGATTTCA TGGGTTGTGT
22690 22700 22710 22720 22730 22740 22750
>< SfaNI
>< RmaI
>< MaeI
>< BsrI
DdeI ><
BfrI ><
CCTTGCTTGG AATACTAGGA ACATTGATGC TACTTCAACT GGTAATTATA ATTATAAATA TAGGTATCTT
22760 22770 22780 22790 22800 22810 22820
>< Sau96I
>< PalI
>< NspIV
> < HindIII
>< HaeIII
>< Eco0109I
>< DraII
>< DdeI
>< Cfr13I
>< BsuRI
>< BsiZI
>< BshI
>< BfrI >< PssI
>< NlaIII >< AsuI>< BsmAI
>< AluI >< Alw26I
BspWI ><
AGACATGGCA AGCTTAGGCC CTTTGAGAGA GACATATCTA ATGTGCCTTT CTCCCCTGAT GGCAAACCTT
22830 22840 22850 22860 22870 22880 22890
>< Tru9I
>< PalI
>< MscI
>< HaeIII
>< EaeI>< MseI
>< Tru9I
>< BsuRI
>< MseI
>< BshI
>< BspMI
>< BalI
BsrI ><
GCACCCCACC TGCTCTTAAT TGTTATTGGC CATTAAATGA TTATGGTTTT TACACCACTA CTGGCATTGG
22900 22910 22920 22930 22940 22950 22960
Sau96I ><
>< PalINspIV ><
> < MspI NspHII ><
>< HaeIII

FIGURE 13.53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>< DsaI
> < HapII Cfr13I ><
>< BsuRISinI ><
>< GdiII BsiZI ><
>< ScaI >< BsaJI
>< RsaI >< Tru9I >< EaeI Bme18I ><
>< Csp6I >< MseI >< Cfr10I AvaII ><
>< AfaI >< DraI >< BshI AsuI ><
CTACCAACCT TACAGAGTTG TAGTACTTTC TTTTGAACTT TTAAATGCAC CGGCCACGGT TTGTGGACCA
22970 22980 22990 23000 23010 23020 23030
>< Tru9I >< RsaI
>< Tru9I >< Csp6I
>< PleI BsrI ><
> < Tru9I >< MseI >< BsrI
> < MseI>< BsrI >< MseI >< HinfI >< AfaI
AAATTATCCA CTGACCTTAT TAAGAACCAG TGTGTCAATT TTAATTTTAA TGGACTCACT GGTACTGGTG
23040 23050 23060 23070 23080 23090 23100
>< Tru9I
>< MseI
>< MboII
>< HpaI
>< HindII
>< HincII
>< PalI
>< HaeIII
>< GdiII
>< EaeI
>< BsuRI TfiI ><
>< BshI HinfI ><
TGTTAACTCC TTCTTCAAAG AGATTTCAAC CATTTCAACA ATTTGGCCGT GATGTTTCTG ATTTCACTGA
23110 23120 23130 23140 23150 23160 23170
> < XhoII
>< TthHB8I
>< TaqI
> < Sau3AI
> < NdeII
> < MflI
> < MboI
> < DpnII
>< DpnI
> < BstYI
> < BspAI > < SspI
>< AlwI >< Bsp143I >< HphI
TTCCGTTCGA GATCCTAAAA CATCTGAAAT ATTAGACATT TCACCTTGCT CTTTTGGGGG TGTAAGTGTA
23180 23190 23200 23210 23220 23230 23240
>< ScrFI
>< MvaI
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< ApyI
>< Tru9I
>< MseI
>< HpaI
>< HindII
>< Eco57I
>< BsgI >< HincII
ATTACACCTG GAACAAATGC TTCATCTGAA GTTGCTGTTC TATATCAAGA TGTTAACTGC ACTGATGTTT
23250 23260 23270 23280 23290 23300 23310
>< Sau3AI
>< NlaIII
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< HinPlI

FIGURE 13. 54

```
          >< BspWI                    >< Hin6I
              >< BspAI                  > < HhaI              PleI ><
 >< SfcI          >< Bsp143I      >< AluI> < CfoI          >< BsrI
CTACAGCAAT TCATGCAGAT CAACTCACAC CAGCTTGGCG CATATATTCT ACTGGAAACA ATGTATTCCA
     23320      23330      23340      23350      23360      23370      23380

                                    >< TthHB8I
                                    >< TaqI
                                   >< SalI
                                   >< RtrI
                                   >< NspI
                             >< EspI  >< NspHI
                             >< DdeI  >< NlaIII
                             >< CelII   >< HindII
                             >< Bpu1102I>< HincII
>< HinfI                     >< AluI    >< AccI
GACTCAAGCA GGCTGTCTTA TAGGAGCTGA GCATGTCGAC ACTTCTTATG AGTGCGACAT TCCTATTGGA
     23390      23400      23410      23420      23430      23440      23450

                                       > < SnaBI
                                            >< ScaI
                                            >< RsaI
                                              >< RmaI
                                          >< MaeII >< MaeI
                                           > < Eco105I
                 >< RmaI                      >< Csp6I
                   >< MaeIII                 > < BsaAI
>< AluI          >< MaeI                        >< AfaI
GCTGGCATTT GTGCTAGTTA CCATACAGTT TCTTTATTAC GTAGTACTAG CCAAAAATCT ATTGTGGCTT
     23460      23470      23480      23490      23500      23510      23520

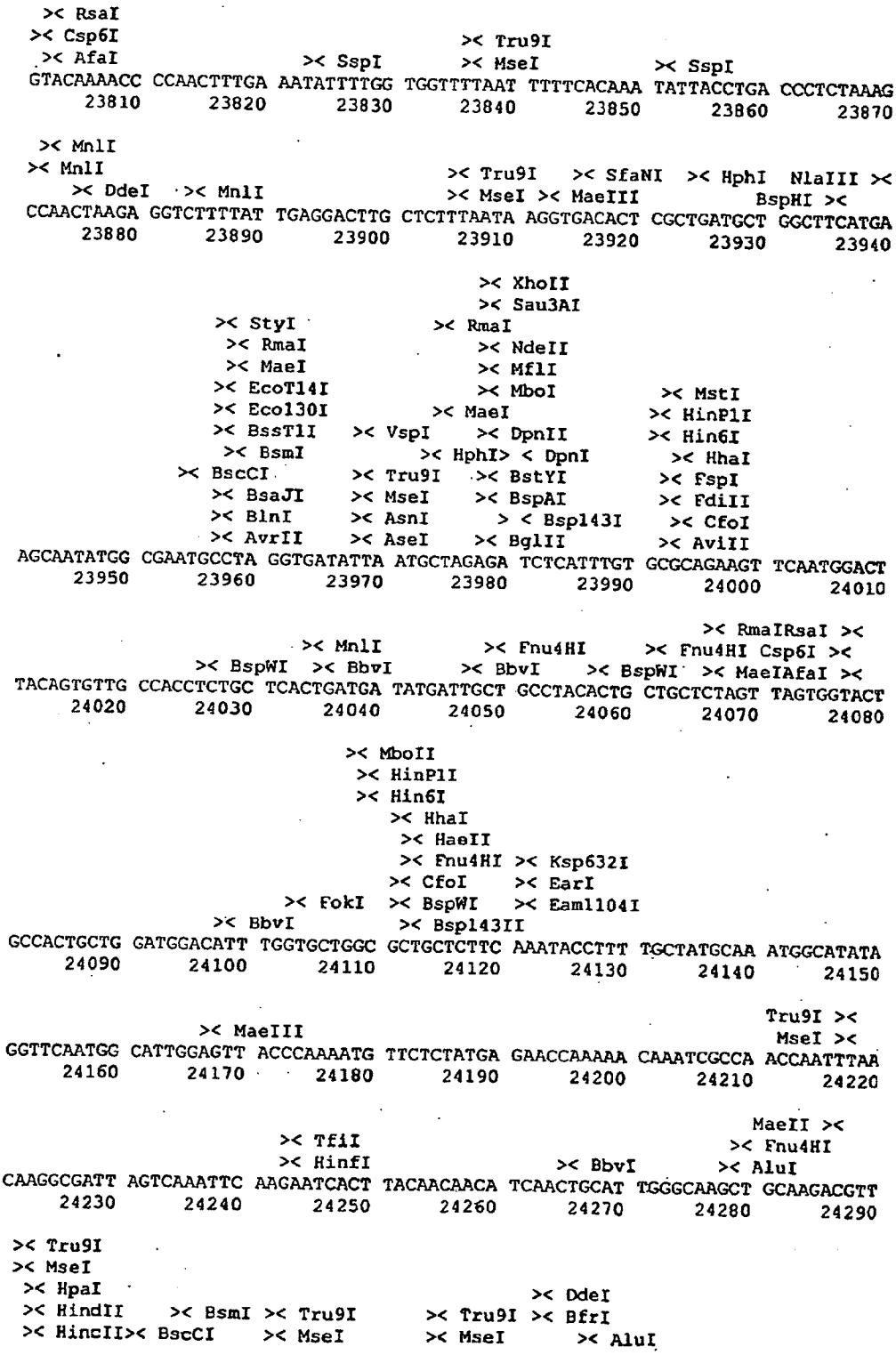
                              >< MunI
ATACTATGTC TTTAGGTGCT GATAGTTCAA TTGCTTACTC TAATAACACC ATTGCTATAC CTACTAACTT
     23530      23540      23550      23560      23570      23580      23590

                                                                  RsaI ><
                                                              >< MnlI
                                                                  Csp6I ><
                    >< SfcI                                         AfaI ><
TTCAATTAGC ATTACTACAG AAGTAATGCC TGTTTCTATG GCTAAAACCT CCGTAGATTG TAATATGTAC
     23600      23610      23620      23630      23640      23650      23660

           > < TfiI
           > < HinfI
      >< AciI                                          > < AluI
ATCTGCGGAG ATTCTACTGA ATGTGCTAAT TTGCTTCTCC AATATGGTAG CTTTTGCACA CAACTAAATC
     23670      23680      23690      23700      23710      23720      23730

>< VneI
    >< SduI
    >< NspII
    >< HgiAI                                    >< PmlI
>< SnoI>< DdeI                    >< Sau3AI      >< PmaCI
    >< Bsp1286I                   >< NdeII      >< MaeII
    >< BmyI                       >< MboI        >< Eco72I
    >< BbvI                          >< DpnI     >< BsaAI
>< ApaLI                             >< Bsp143I  >< BbrPI
>< Alw44I                         >< DpnII >< AlwI
    >< Alw21I       >< Fnu4HI  >< BspAI    >< AflIII
GTGCACTCTC AGGTATTGCT GCTGAACAGG ATCGCAACAC ACGTGAAGTG TTCGCTCAAG TCAAACAAAT
     23740      23750      23760      23770      23780      23790      23800
```

FIGURE 13.55


>< RsaI
>< Csp6I
>< AfaI
>< SspI
>< Tru9I
>< MseI
>< SspI
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23810 23820 23830 23840 23850 23860 23870
>< MnlI
>< MnlI
>< DdeI
>< MnlI
>< Tru9I
>< MseI
>< SfaNI
>< MaeIII
>< HphI
NlaIII ><
BspHI ><
CCAACTAAGA GGTCTTTTAT TGAGGACTTG CTCTTTAATA AGGTGACACT CGCTGATGCT GGCTTCATGA
23880 23890 23900 23910 23920 23930 23940
>< XhoII
>< Sau3AI
>< StyI
>< RmaI
>< RmaI
>< NdeII
>< MaeI
>< MflI
>< EcoT14I
>< MboI
>< MstI
>< Eco130I
>< MaeI
>< HinP1I
>< BssT1I
>< VspI
>< DpnII
>< Hin6I
>< BsmI
>< HphI> < DpnI
>< HhaI
>< BscCI
>< Tru9I
>< BstYI
>< FspI
>< BsaJI
>< MseI
>< BspAI
>< FdiII
>< BlnI
>< AsnI
> < Bsp143I
>< CfoI
>< AvrII
>< AseI
>< BglII
>< AviII
AGCAATATGG CGAATGCCTA GGTGATATTA ATGCTAGAGA TCTCATTTGT GCGCAGAAGT TCAATGGACT
23950 23960 23970 23980 23990 24000 24010
>< RmaIRsaI ><
>< MnlI
>< Fnu4HI
>< Fnu4HI Csp6I ><
>< BspWI
>< BbvI
>< BbvI
>< BspWI
>< MaeIAfaI ><
TACAGTGTTG CCACCTCTGC TCACTGATGA TATGATTGCT GCCTACACTG CTGCTCTAGT TAGTGGTACT
24020 24030 24040 24050 24060 24070 24080
>< MboII
>< HinP1I
>< Hin6I
>< HhaI
>< HaeII
>< Fnu4HI
>< Ksp632I
>< CfoI
>< EarI
>< FokI
>< BspWI
>< Eam1104I
>< BbvI
>< Bsp143II
GCCACTGCTG GATGGACATT TGGTGCTGGC GCTGCTCTTC AAATACCTTT TGCTATGCAA ATGGCATATA
24090 24100 24110 24120 24130 24140 24150
Tru9I ><
MseI ><
>< MaeIII
GGTTCAATGG CATTGGAGTT ACCCAAAATG TTCTCTATGA GAACCAAAAA CAAATCGCCA ACCAATTTAA
24160 24170 24180 24190 24200 24210 24220
MaeII ><
>< Fnu4HI
>< TfiI
>< HinfI
>< BbvI
>< AluI
CAAGGCGATT AGTCAAATTC AAGAATCACT TACAACAACA TCAACTGCAT TGGGCAAGCT GCAAGACGTT
24230 24240 24250 24260 24270 24280 24290
>< Tru9I
>< MseI
>< HpaI
>< HindII
>< HincII
>< BsmI
>< BscCI
>< Tru9I
>< MseI
>< DdeI
>< Tru9I
>< BfrI
>< MseI
>< AluI

FIGURE 13. 56

```
GTTAACCAGA ATGCTCAAGC ATTAAACACA CTTGTTAAAC AACTTAGCTC TAATTTTGGT GCAATTTCAA
     24300      24310      24320      24330      24340      24350      24360

                        >< ThaI
                        >< SpoI
                        >< NruI
                        >< MvnI
                        >< BstUI       >< TthHB8I
                        >< Bsp68I      >< TaqI      >< RsaI
              >< EcoRV  >< Bsp50I      >< MnlI     >< Csp6I         >< Tru9I
              >< Eco32I >< AccII >< MnlI     >< AciI>< AfaI         >< MseI
GTGTGCTAAA TGATATCCTT TCGCGACTTG ATAAAGTCGA GGCGGAGGTA CAAATTGACA GGTTAATTAC
     24370      24380      24390      24400      24410      24420      24430

                           >< MaeIII >< BbvI         >< Fnu4HI   BbvI ><
AGGCAGACTT CAAAGCCTTC AAACCTATGT AACACAACAA CTAATCAGGG CTGCTGAAAT CAGGGCTTCT
     24440      24450      24460      24470      24480      24490      24500

          >< Fnu4HI                                          >< HindII
      >< BspWI             >< DdeI                          >< HincII
GCTAATCTTG CTGCTACTAA AATGTCTGAG TGTGTTCTTG GACAATCAAA AAGAGTTGAC TTTTGTGGAA
     24510      24520      24530      24540      24550      24560      24570

                                                             > < NspI
                                                             > < NspHI
                                                             > < NlaIII
                                                            >< MaeIII
                                                 >< NlaIII        >< MaeII
                                                >< MboII         >< FokI
                                      >< Fnu4HI  >< BbsI         BsaAI ><
                                         >< AciI>< BbvI       >< AflIII
AGGGCTACCA CCTTATGTCC TTCCCACAAG CAGCCCCGCA TGGTGTTGTC TTCCTACATG TCACGTATGT
     24580      24590      24600      24610      24620      24630      24640

       >< ScrFI
       >< MvaI
      >< EcoRII
        >< Ecl136I
        >< BstOI
        >< BstNI              >< HinP1I
    >< MnlI >< BslI           >< Hin6I
      >< DsaV>< BsiYI            >< HhaI
        >< BsiLI                  >< HaeII
      >< BsaJI>< HphI            >< CfoI          >< NlaIII
        >< ApyI                   >< Bsp143II >< BspHI                EcoNI ><
GCCATCCCAG GAGAGGAACT TCACCACAGC GCCAGCAATT TGTCATGAAG GCAAAGCATA CTTCCCTCGT
     24650      24660      24670      24680      24690      24700      24710

     >< MnlI
><  BslI          >< Tru9I
><  BsiYI         >< MseI              >< MnlI
GAAGGTGTTT TTGTGTTTAA TGGCACTTCT TGGTTTATTA CACAGAGGAA CTTCTTTTCT CCACAAATAA
     24720      24730      24740      24750      24760      24770      24780

                        >< DdeI                              >< Tru9I
                           >< BsmAI                             >< SfaNI
    >< SfcI                >< Alw26I                         >< MseIAlwI ><
TTACTACAGA CAATACATTT GTCTCAGGAA ATTGTGATGT CGTTATTGGC ATCATTAACA ACACAGTTTA
     24790      24800      24810      24820      24830      24840      24850

><  Sau3AI
><  NdeII
```

FIGURE 13.57

```
><MboI          ><PleI                                  > < ScaI
><DpnII       ><MnlI          > < Ksp632I               > < RsaI
  ><DpnI      ><DdeI  ><HinfI                       ><MboII
><BspAI        ><BspWI       > < Eam1104I           ><Csp6I
  ><Bsp143I       ><AluI     > < EarI   > < AluI   > < AfaI   > < HphI
TGATCCTCTG CAACCTGAGC TTGACTCATT CAAAGAAGAG CTGGACAAGT ACTTCAAAAA TCATACATCA
     24860      24870      24880      24890      24900      24910      24920

       ><Sau3AI
       ><NdeII
       ><MboI
       ><MamI
       ><DpnII
          ><DpnI
       ><BspAI
          ><Bsp143I
       ><BsiBI                  ><Tru9I         ><HindII
       ><BsaBI                  ><MseI          ><HincII          AciI ><
CCAGATGTTG ATCTTGGCGA CATTTCAGGC ATTAACGCTT CTGTCGTCAA CATTCAAAAA GAAATTGACC
     24930      24940      24950      24960      24970      24980      24990

                        ><Tru9I
                              > < TfiI
            ><MnlI       ><SwaI
     ><EcoNI            ><MseI
       ><BslI                 > < HinfI
><MnlI><BsiYI            ><DraI
GCCTCAATGA GGTCGCTAAA AATTTAAATG AATCACTCAT TGACCTTCAA GAATTGGGAA AATATGAGCA
     25000      25010      25020      25030      25040      25050      25060

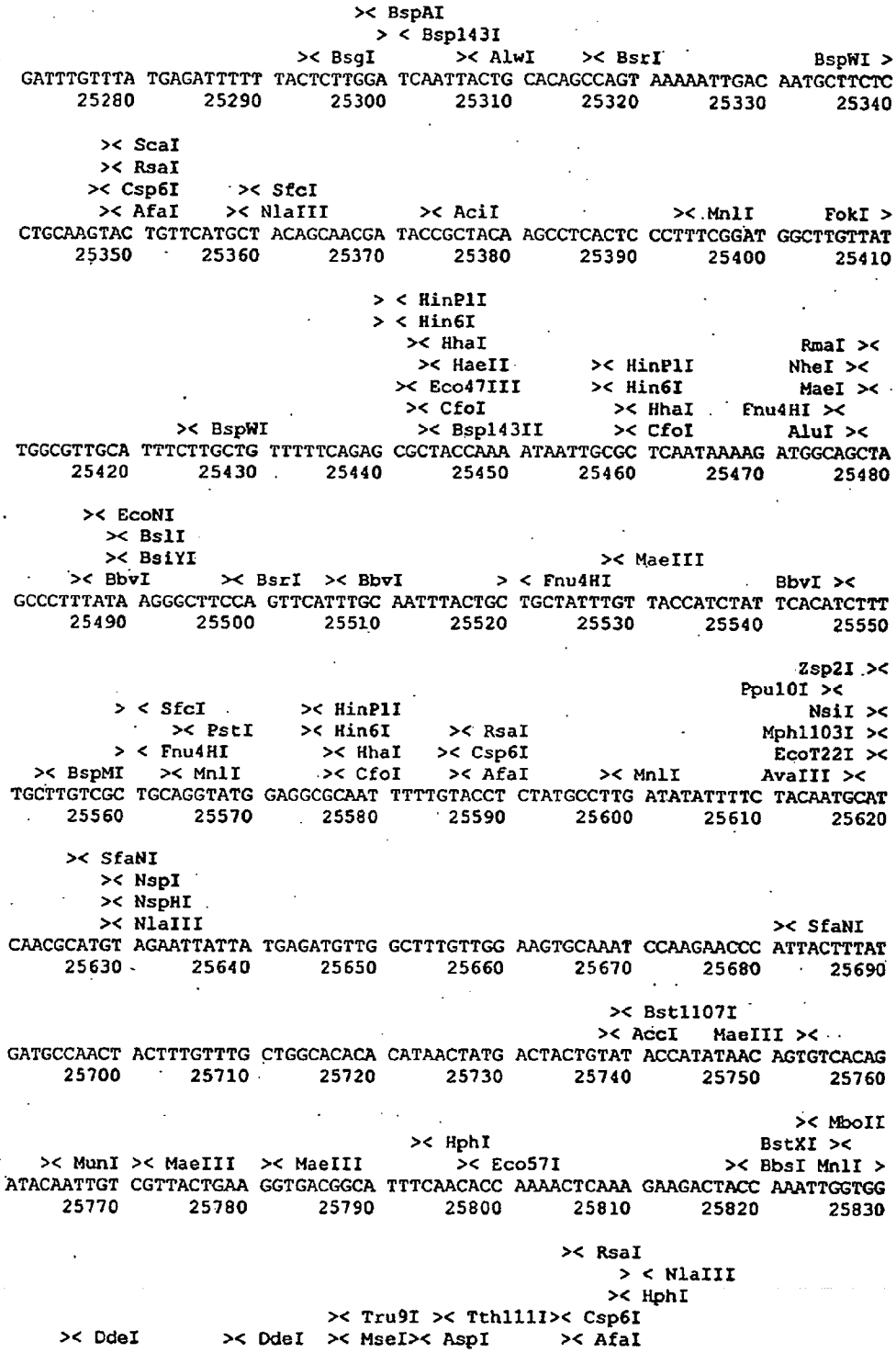
             ><StyI
            ><PalI
            ><HaeIII
             ><Eco T14I
             ><Eco130I
            ><BsuRI
             ><BssT1I                                              NlaIII ><
    ><Tru9I><BshI                                                  MaeIII ><
    ><MseI  ><BsaJI                                                      ><BstXI
ATATATTAAA TGGCCTTGGT ATGTTTGGCT CGGCTTCATT GCTGGACTAA TTGCCATCGT CATGGTTACA
     25070      25080      25090      25100      25110      25120      25130

                                                 > < SphI
                                                 > < PaeI
                  ><SpeI                         > < NspI
                   > < RmaI                      > < NspHI
                  ><NlaIII                       > < NlaIII
                    > < MaeI                  ><MnlI><BbvI Fnu4HI ><
ATCTTGCTTT GTTGCATGAC TAGTTGTTGC AGTTGCCTCA AGGGTGCATG CTCTTGTGGT TCTTGCTGCA
     25140      25150      25160      25170      25180      25190      25200

                        ><FokI
                      ><DdeI
><MnlI ><PleI><HinfI  ><BsrI
AGTTTGATGA GGATGACTCT GAGCCAGTTC TCAAGGGTGT CAAATTACAT TACACATAAA CGAACTTATG
     25210      25220      25230      25240      25250      25260      25270

                           ><Sau3AI
                           ><NdeII
                           ><MboI
                           ><DpnII
                             > < DpnI
```

FIGURE 13.58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> < Bsp143I
>< BsgI >< AlwI >< BsrI BspWI >
GATTTGTTTA TGAGATTTTT TACTCTTGGA TCAATTACTG CACAGCCAGT AAAAATTGAC AATGCTTCTC
25280 25290 25300 25310 25320 25330 25340
>< ScaI
>< RsaI
>< Csp6I >< SfcI
>< AfaI >< NlaIII >< AciI >< MnlI FokI >
CTGCAAGTAC TGTTCATGCT ACAGCAACGA TACCGCTACA AGCCTCACTC CCTTTCGGAT GGCTTGTTAT
25350 25360 25370 25380 25390 25400 25410
> < HinPlI
> < Hin6I
>< HhaI
>< HaeII >< HinPlI NheI ><
>< Eco47III >< Hin6I MaeI ><
>< CfoI >< HhaI Fnu4HI ><
>< BspWI >< Bsp143II >< CfoI AluI ><
TGGCGTTGCA TTTCTTGCTG TTTTTCAGAG CGCTACCAAA ATAATTGCGC TCAATAAAAG ATGGCAGCTA
25420 25430 25440 25450 25460 25470 25480
>< EcoNI
>< BslI
>< BsiYI
>< MaeIII
>< BbvI >< BsrI >< BbvI > < Fnu4HI BbvI ><
GCCCTTTATA AGGGCTTCCA GTTCATTTGC AATTTACTGC TGCTATTTGT TACCATCTAT TCACATCTTT
25490 25500 25510 25520 25530 25540 25550
Zsp2I ><
Ppu10I ><
> < SfcI >< HinPlI NsiI ><
>< PstI >< Hin6I >< RsaI Mph1103I ><
> < Fnu4HI >< HhaI >< Csp6I EcoT22I ><
>< BspMI >< MnlI >< CfoI >< AfaI >< MnlI AvaIII ><
TGCTTGTCGC TGCAGGTATG GAGGCGCAAT TTTTGTACCT CTATGCCTTG ATATATTTTC TACAATGCAT
25560 25570 25580 25590 25600 25610 25620
>< SfaNI
>< NspI
>< NspHI
>< NlaIII
>< SfaNI
CAACGCATGT AGAATTATTA TGAGATGTTG GCTTTGTTGG AAGTGCAAAT CCAAGAACCC ATTACTTTAT
25630 25640 25650 25660 25670 25680 25690
>< Bst1107I
>< AccI MaeIII ><
GATGCCAACT ACTTTGTTTG CTGGCACACA CATAACTATG ACTACTGTAT ACCATATAAC AGTGTCACAG
25700 25710 25720 25730 25740 25750 25760
>< MboII
>< HphI BstXI ><
>< MunI >< MaeIII >< MaeIII >< Eco57I >< BbsI MnlI >
ATACAATTGT CGTTACTGAA GGTGACGGCA TTTCAACACC AAAACTCAAA GAAGACTACC AAATTGGTGG
25770 25780 25790 25800 25810 25820 25830
>< RsaI
> < NlaIII
>< HphI
>< Tru9I >< Tth111I >< Csp6I
>< DdeI >< DdeI >< MseI >< AspI >< AfaI

FIGURE 13.59

```
TTATTCTGAG GATAGGCACT CAGGTGTTAA AGACTATGTC GTTGTACATG GCTATTTCAC CGAAGTTTAC
     25840      25850      25860      25870      25880      25890      25900

                                                                  Tru9I ><
        > < HinfI>< PleI                  >< BsrI                  MseI ><
     >< AluI >< AccI       >< SfcI  >< AlwNI       >< MboII        HindIII >
TACCAGCTTG AGTCTACACA AATTACTACA GACACTGGTA TTGAAAATGC TACATTCTTC ATCTTTAACA
     25910      25920      25930      25940      25950      25960      25970

                                      > < TthHB8I
     >< Tru9I                         > < TaqI       >< Ksp632I
     >< MseI                          > < MboII      >< EarI BspWI ><
 >< AluI                           >< Eco57I          >< Eam1104I AlwI ><
AGCTTGTTAA AGACCCACCG AATGTGCAAA TACACACAAT CGACGGCTCT TCAGGAGTTG CTAATCCAGC
     25980      25990      26000      26010      26020      26030      26040

   >< XhoII
   >< Sau3AI
     >< NlaIV
   >< NdeII
   >< MflI
   >< MboI
   >< DpnII
     >< DpnI
   >< BstYI
   >< BstI
   >< BspAI
     >< Bsp143I                                                      RsaI ><
     >< BscBI                        >< RmaI                        Csp6I ><
   >< BamHI >< AlwI                  >< MaeI                         AfaI ><
AATGGATCCA ATTTATGATG AGCCGACGAC GACTACTAGC GTGCCTTTGT AAGCACAAGA AAGTGAGTAC
     26050      26060      26070      26080      26090      26100      26110

                                      > < Tru9I
                                    >< RsaI
                                       > < MseI
                                     >< MboII
       > < RsaI                      >< MaeII            >< RsaI
      >< Csp6I                     >< Csp6I     >< Tru9I >< Csp6I
       > < AfaI                    >< AfaI      >< MseI   >< AfaI
GAACTTATGT ACTCATTCGT TTCGGAAGAA ACAGGTACGT TAATAGTTAA TAGCGTACTT CTTTTTCTTG
     26120      26130      26140      26150      26160      26170      26180

                                                 >< TthHB8I
                                                 >< TaqI
                 >< RmaI                      >< HinP1I          > < RsaI
                   > < MaeIII                 >< Hin6I          Fnu4HI ><
                 >< MaeI   >< RmaI              >< HhaI         >< Csp6I
                 >< FokI   >< MaeI              >< CfoI >< BbvI  > < AfaI
CTTTCGTGGT ATTCTTGCTA GTCACACTAG CCATCCTTAC TGCGCTTCGA TTGTGTGCGT ACTGCTGCAA
     26190      26200      26210      26220      26230      26240      26250

                                                      >< Tru9I
     >< Tru9I                                       >< ThaI
     >< MseI                                        >< MvnI
>< SspI >< MaeII                                         >< MseI
      >< HpaI                                       >< BstUI          Ksp632I >
      >< HindII                          >< MaeII   >< Bsp50I   >< MboII EarI >
      >< HincII                             >< AccI >< AccII        Eam1104I >
TATTGTTAAC GTGAGTTTAG TAAAACCAAC GGTTTACGTC TACTCGCGTG TTAAAAATCT GAACTCTTCT
     26260      26270      26280      26290      26300      26310      26320
```

FIGURE 13.60

```
            >< Sau3AI
            >< NdeII
            >< MboI
            >< DpnII
          >< MboII>< DpnI
        >< XmnI >< BspAI> < Eco57I                                    >< Tru9I
        >< Asp700I>< Bsp143I                                          >< MseI
GAAGGAGTTC CTGATCTTCT GGTCTAAACG AACTAACTAT TATTATTATT CTGTTTGGAA CTTTAACATT
     26330      26340      26350      26360      26370      26380      26390

                                                        >< ScrFI
                                                        >< MvaI
                                                      >< EcoRII
                                                        >< Ecl136I
                                                      >< DsaV  NlaIV ><
                          >< RsaI                       >< BstOI
                               >< MnlI          >< Tru9I  >< BstNI   RmaI ><
                          >< Csp6I              >< MseI   >< BsiLI   MaeI ><
          > < NlaIII     >< AfaI          > < AluI       >< ApyIBscBI ><
GCTTATCATG GCAGACAACG GTACTATTAC CGTTGAGGAG CTTAAACAAC TCCTGGAACA ATGGAACCTA
     26400      26410      26420      26430      26440      26450      26460

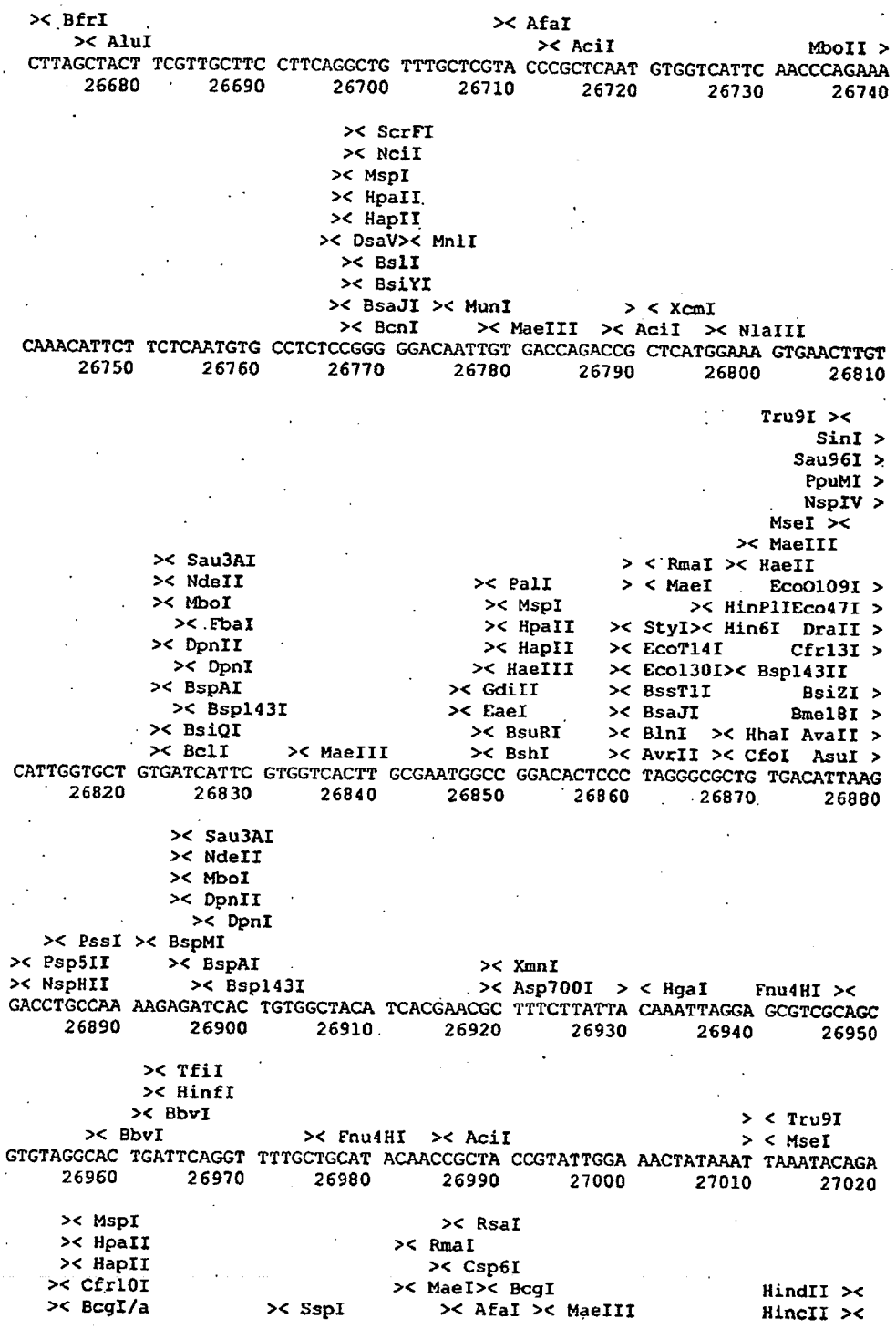
                            >< ScrFI
                        >< RmaI
                            >< MvaI
                        >< MaeI
                            >< EcoRII
                              >< Ecl136I
                            >< DsaV
                              >< BstOI
                              >< BstNI
                              >< BsiLI
                              >< ApyI  >< MaeIII
GTAATAGGTT TCCTATTCCT AGCCTGGATT ATGTTACTAC AATTTGCCTA TTCTAATCGG AACAGGTTTT
     26470      26480      26490      26500      26510      26520      26530

                                          >< PalI
                                          >< MscI
                                       >< MnlI  >< MaeIII
                                          >< HaeIII
                                       >< EaeI
                                          >< BsuRI
                                           >< BsrI
  >< RsaI                              >< BspWI
 >< Csp6I    >< HindIII                   >< BshI
  >< AfaI      >< AluI                    >< BalI          >< BbvI Fnu4HI ><
TGTACATAAT AAAGCTTGTT TTCCTCTGGC TCTTGTGGCC AGTAACACTT GCTTGTTTTG TGCTTGCTGC
     26540      26550      26560      26570      26580      26590      26600

            >< VspI
            >< Tru9I
            >< MseI                 >< HphI
   >< SfcI  >< AsnI              >< BsrI
  >< AccI   >< AseI>< MaeIII>< AciI
TGTCTACAGA ATTAATTGGG TGACTGGCGG GATTGCGATT GCAATGGCTT GTATTGTAGG CTTGATGTGG
     26610      26620      26630      26640      26650      26660      26670

><  EspI
      >< Eco57I
><  DdeI
><  CelII                                  >< RsaI
><  Bpu1102I                               >< Csp6I
```

FIGURE 13.61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>< AluI
>< AciI
MboII >
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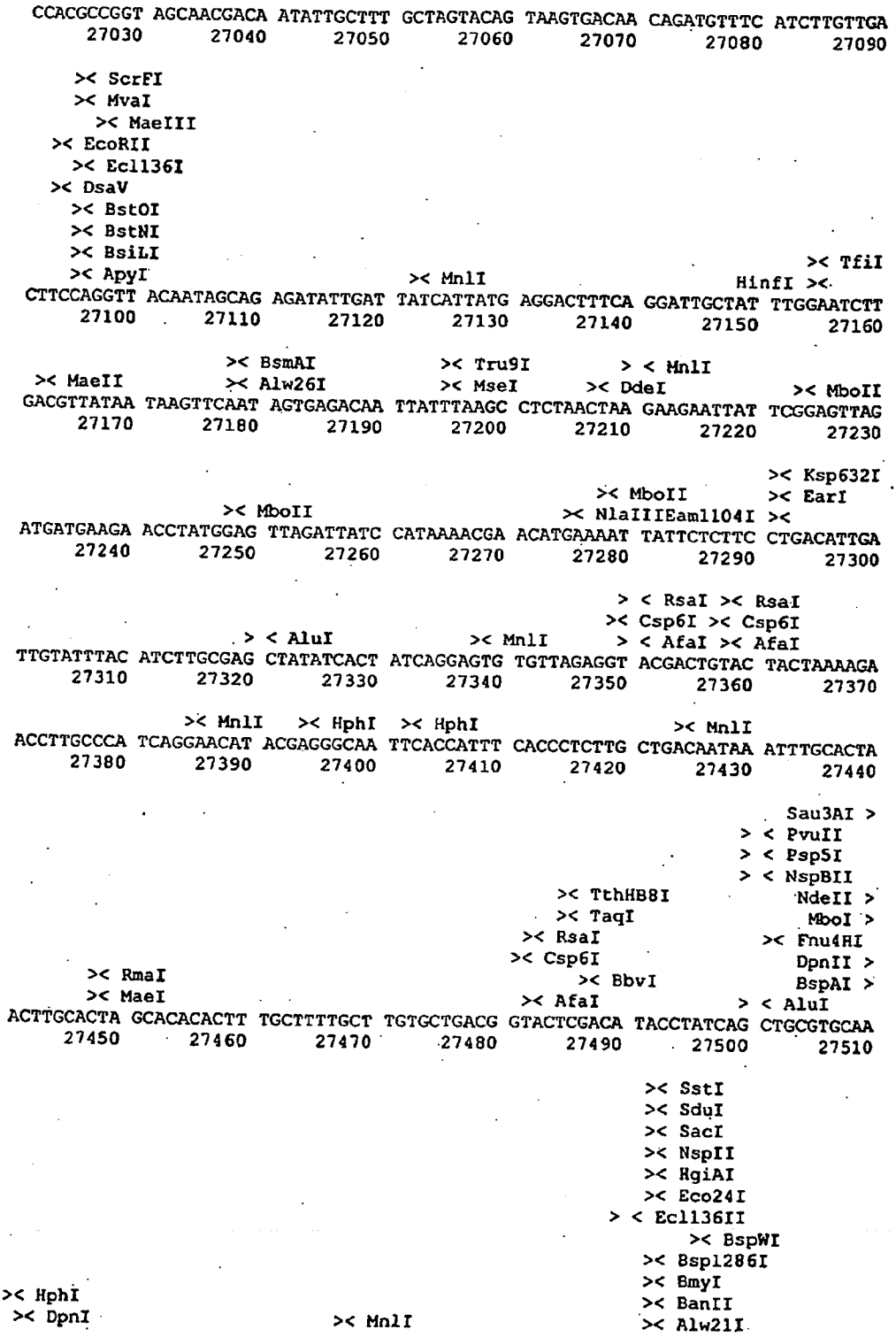
26680 26690 26700 26710 26720 26730 26740
>< ScrFI
>< NciI
>< MspI
>< HpaII
>< HapII
>< DsaV>< MnlI
>< BslI
>< BsiYI
>< BsaJI >< MunI > < XcmI
>< BcnI >< MaeIII >< AciI >< NlaIII
CAAACATTCT TCTCAATGTG CCTCTCCGGG GGACAATTGT GACCAGACCG CTCATGGAAA GTGAACTTGT
26750 26760 26770 26780 26790 26800 26810
Tru9I ><
SinI >
Sau96I >
PpuMI >
NspIV >
MseI ><
>< MaeIII
>< Sau3AI
> < RmaI >< HaeII
>< NdeII
>< PalI
> < MaeI
Eco0109I >
>< MboI
>< MspI
>< HinP1IEco47I >
>< FbaI
>< HpaII
>< StyI>< Hin6I DraII >
>< DpnII
>< HapII
>< EcoT14I
Cfr13I >
>< DpnI
>< HaeIII
>< Eco130I>< Bsp143II
>< BspAI
>< GdiII
>< BssT1I
BsiZI >
>< Bsp143I
>< EaeI
>< BsaJI
Bme18I >
>< BsiQI
>< BsuRI
>< BlnI >< HhaI AvaII >
>< BclI >< MaeIII
>< BshI
>< AvrII >< CfoI AsuI >
CATTGGTGCT GTGATCATTC GTGGTCACTT GCGAATGGCC GGACACTCCC TAGGGCGCTG TGACATTAAG
26820 26830 26840 26850 26860 26870 26880
>< Sau3AI
>< NdeII
>< MboI
>< DpnII
>< DpnI
>< PssI >< BspMI
>< Psp5II
>< BspAI
>< XmnI
>< NspHII
>< Bsp143I
>< Asp700I > < HgaI
Fnu4HI ><
GACCTGCCAA AAGAGATCAC TGTGGCTACA TCACGAACGC TTTCTTATTA CAAATTAGGA GCGTCGCAGC
26890 26900 26910 26920 26930 26940 26950
>< TfiI
>< HinfI
>< BbvI
> < Tru9I
>< BbvI
>< Fnu4HI >< AciI
> < MseI
GTGTAGGCAC TGATTCAGGT TTTGCTGCAT ACAACCGCTA CCGTATTGGA AACTATAAAT TAAATACAGA
26960 26970 26980 26990 27000 27010 27020
>< MspI
>< RsaI
>< HpaII
>< RmaI
>< HapII
>< Csp6I
>< Cfr10I
>< MaeI>< BcgI
HindII ><
>< BcgI/a
>< SspI
>< AfaI >< MaeIII
HincII ><

FIGURE 13.62


CCACGCCGGT AGCAACGACA ATATTGCTTT GCTAGTACAG TAAGTGACAA CAGATGTTTC ATCTTGTTGA
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>< ScrFI
>< MvaI
>< MaeIII
>< EcoRII
>< Ecl136I
>< DsaV
>< BstOI
>< BstNI
>< BsiLI
>< ApyI
>< MnlI
>< TfiI
HinfI ><
CTTCCAGGTT ACAATAGCAG AGATATTGAT TATCATTATG AGGACTTTCA GGATTGCTAT TTGGAATCTT
27100 27110 27120 27130 27140 27150 27160
>< BsmAI
>< Tru9I
> < MnlI
>< MaeII
>< Alw26I
>< MseI
>< DdeI
>< MboII
GACGTTATAA TAAGTTCAAT AGTGAGACAA TTATTTAAGC CTCTAACTAA GAAGAATTAT TCGGAGTTAG
27170 27180 27190 27200 27210 27220 27230
>< Ksp632I
>< MboII
>< EarI
>< MboII
>< NlaIIIEam1104I ><
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>< MnlI
> < AfaI >< AfaI
TTGTATTTAC ATCTTGCGAG CTATATCACT ATCAGGAGTG TGTTAGAGGT ACGACTGTAC TACTAAAAGA
27310 27320 27330 27340 27350 27360 27370
>< MnlI
>< HphI
>< HphI
>< MnlI
ACCTTGCCCA TCAGGAACAT ACGAGGGCAA TTCACCATTT CACCCTCTTG CTGACAATAA ATTTGCACTA
27380 27390 27400 27410 27420 27430 27440
Sau3AI >
> < PvuII
> < Psp5I
> < NspBII
>< TthHB8I
NdeII >
>< TaqI
MboI >
>< RsaI
>< Fnu4HI
>< Csp6I
DpnII >
>< RmaI
>< BbvI
BspAI >
>< MaeI
>< AfaI
> < AluI
ACTTGCACTA GCACACACTT TGCTTTTGCT TGTGCTGACG GTACTCGACA TACCTATCAG CTGCGTGCAA
27450 27460 27470 27480 27490 27500 27510
>< SstI
>< SduI
>< SacI
>< NspII
>< HgiAI
>< Eco24I
> < Ecl136II
>< BspWI
>< Bspl286I
>< BmyI
>< BanII
>< Alw21I
>< HphI
>< DpnI
>< MnlI

FIGURE 13. 63

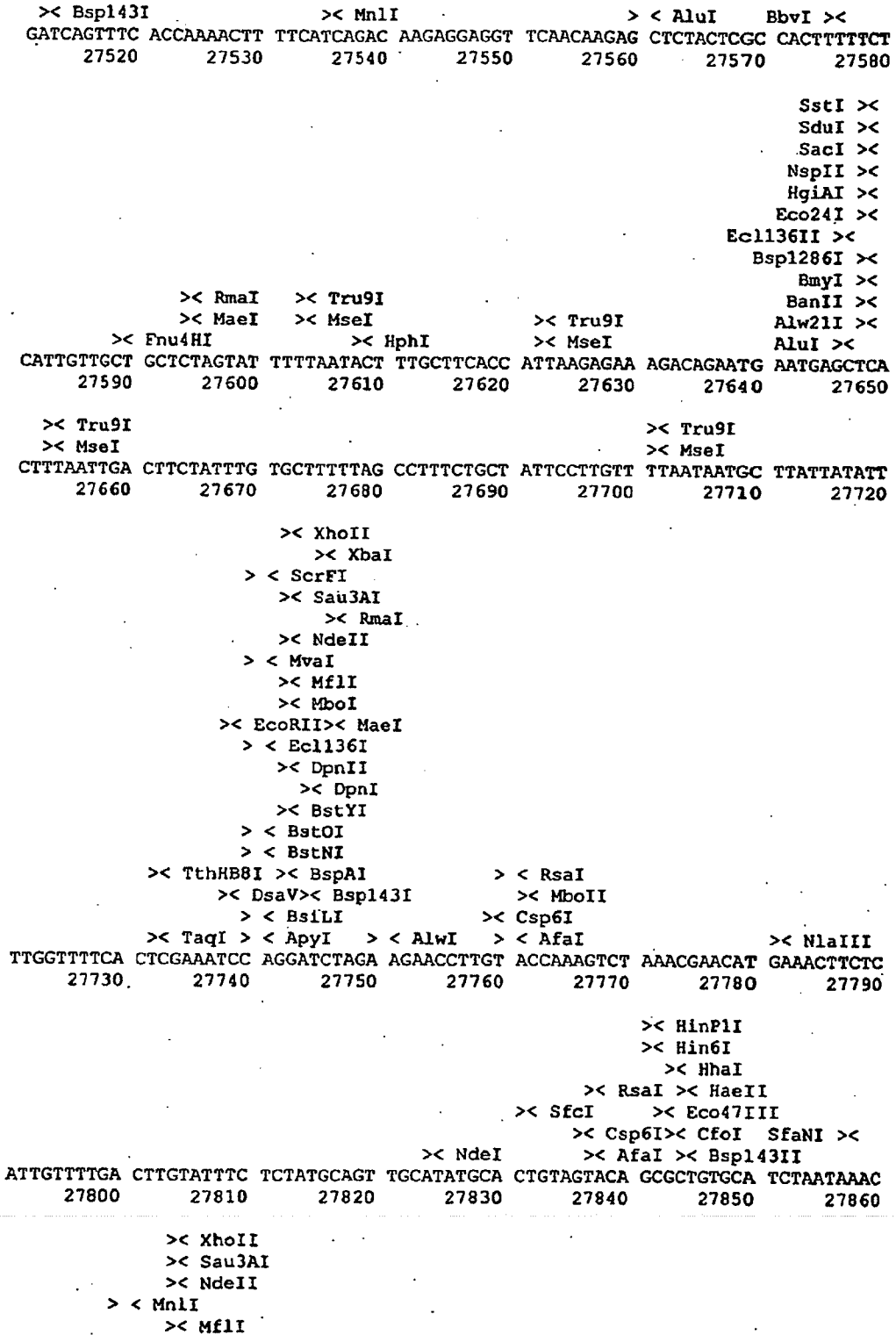

>< Bsp143I
>< MnlI
> < AluI
BbvI ><
GATCAGTTTC ACCAAAACTT TTCATCAGAC AAGAGGAGGT TCAACAAGAG CTCTACTCGC CACTTTTTCT
27520 27530 27540 27550 27560 27570 27580
SstI ><
SduI ><
SacI ><
NspII ><
HgiAI ><
Eco24I ><
Ecl136II ><
Bsp1286I ><
BmyI ><
BanII ><
Alw21I ><
AluI ><
>< RmaI
>< MaeI
>< Tru9I
>< MseI
>< Tru9I
>< MseI
>< Fnu4HI
>< HphI
CATTGTTGCT GCTCTAGTAT TTTTAATACT TTGCTTCACC ATTAAGAGAA AGACAGAATG AATGAGCTCA
27590 27600 27610 27620 27630 27640 27650
>< Tru9I
>< MseI
>< Tru9I
>< MseI
CTTTAATTGA CTTCTATTTG TGCTTTTTAG CCTTTCTGCT ATTCCTTGTT TTAATAATGC TTATTATATT
27660 27670 27680 27690 27700 27710 27720
>< XhoII
>< XbaI
> < ScrFI
>< Sau3AI
>< RmaI
>< NdeII
> < MvaI
>< MflI
>< MboI
>< EcoRII>< MaeI
> < Ecl136I
>< DpnII
>< DpnI
>< BstYI
> < BstOI
> < BstNI
>< TthHB8I >< BspAI
> < RsaI
>< DsaV>< Bsp143I
>< MboII
> < BsiLI
>< Csp6I
>< TaqI > < ApyI
> < AlwI
> < AfaI
>< NlaIII
TTGGTTTTCA CTCGAAATCC AGGATCTAGA AGAACCTTGT ACCAAAGTCT AAACGAACAT GAAACTTCTC
27730 27740 27750 27760 27770 27780 27790
>< HinP1I
>< Hin6I
>< HhaI
>< RsaI >< HaeII
>< SfcI
>< Eco47III
>< Csp6I>< CfoI SfaNI ><
>< NdeI
>< AfaI >< Bsp143II
ATTGTTTTGA CTTGTATTTC TCTATGCAGT TGCATATGCA CTGTAGTACA GCGCTGTGCA TCTAATAAAC
27800 27810 27820 27830 27840 27850 27860
>< XhoII
>< Sau3AI
>< NdeII
> < MnlI
>< MflI

FIGURE 13.64

```
            >< MboI
            >< DpnII
              >< DpnI      >< RsaI
            >< BstYI   >< MboII
     >< NlaIII>< BspAI      >< Csp6I >< RmaI
        >< AlwI >< Bsp143I   >< AfaI >< MaeI
CTCATGTGCT TGAAGATCCT TGTAAGGTAC AACACTAGGG GTAATACTTA TAGCACTGCT TGGCTTTGTG
    27870      27880      27890      27900      27910      27920      27930

 >< SduI
 >< RmaI
 >< NspII
  >< MaeI
 >< HgiAI
 >< Bsp1286I                                          >< NspI
 >< BmyI                                              >< NspHI
 >< Alw21I                                            >< NlaIII  >< MaeIII
CTCTAGGAAA GGTTTTACCT TTTCATAGAT GGCACACTAT GGTTCAAACA TGCACACCTA ATGTTACTAT
    27940      27950      27960      27970      27980      27990      28000

        > < XhoII
        > < Sau3AI > < Van91I                    >< RsaI
                >< PvuII                         >< NlaIV
                >< Psp5I                           >< KpnI >< NlaIII
        > < NdeII  > < PflMI                    >< Eco64I      >< MaeIII
        > < MflI>< NspBII                        >< Csp6I>< HphI
        > < DpnII          >< HinP1I             >< BscBI      >< EcoO65I
           >< Bsp143I      >< Hin6I            >< BanI >< BspHI
        > < BstYI  > < BslI >< HhaI  >< RmaI   >< Asp718      >< Eco91I
        > < BspAI  > < BsiYI>< CfoI  >< MaeI     >< AfaI      >< BstPI
        > < MboI>< AluI>< BspWI  >< BspWI      >< AccBlI      >< BstEII
    >< AlwI >< DpnI > < AccB7I        >< AluI    >< Acc65I     >< BbvI
CAACTGTCAA GATCCAGCTG GTGGTGCGCT TATAGCTAGG TGTTGGTACC TTCATGAAGG TCACCAAACT
    28010      28020      28030      28040      28050      28060      28070

                                                                  >< SinI
                                                                  >< Sau96I
                                                                  >< NspIV
                                                               NspHII ><
                                                                 NlaIV ><
                                                                  >< Eco47I
                                                                  >< Cfr13I
                 >< RsaI                                          >< BsiZI
 >< Fnu4HI     >< MaeII                                         BscBI ><
    >< Esp3I     >< Csp6I      >< Tru9I                          >< Bme18I
    >< BsmAI      >< BsmBI     >< MseI           >< Tru9I        >< AvaII
    >< Alw26I     >< AfaI       >< DraI          >< MseI         >< AsuI
GCTGCATTTA GAGACGTACT TGTTGTTTTA AATAAACGAA CAAATTAAAA TGTCTGATAA TGGACCCCAA
    28080      28090      28100      28110      28120      28130      28140

                                  >< SinI
                                  >< Sau96I
                                  >< NspIV
                                   >< NspHII
                                     >< NlaIV
                                  >< Eco47I
                                  >< Cfr13I
                 >< SduI          >< BsiZI
                 >< NspII           >< BscBI
                 >< Bsp1286I      >< Bme18I
                 >< BmyI          >< AvaII  >< TfiI
       >< MaeII      >< AciI      >< AsuI   >< HinfI          >< MnlI
```

FIGURE 13.65

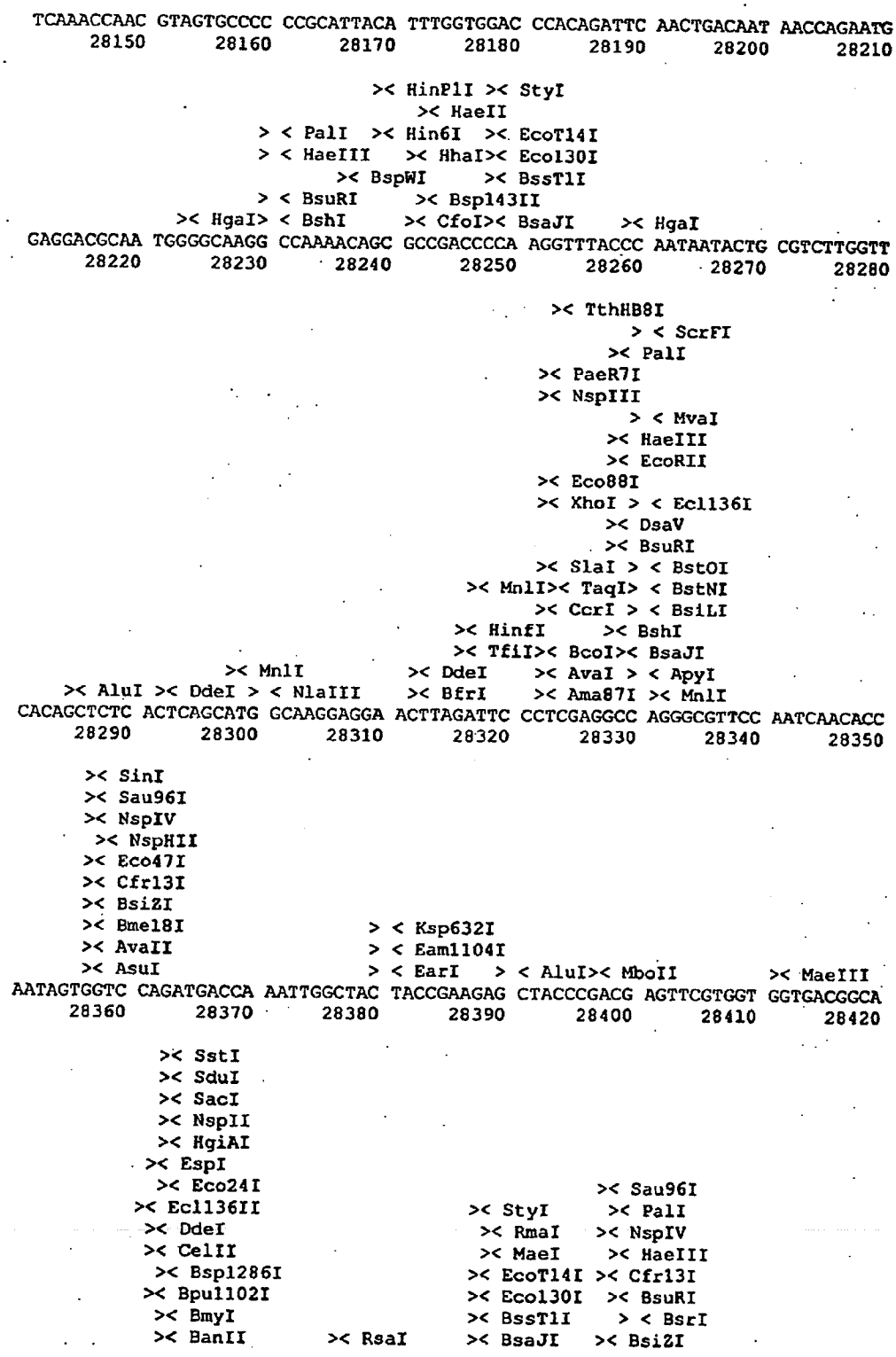

TCAAACCAAC GTAGTGCCCC CCGCATTACA TTTGGTGGAC CCACAGATTC AACTGACAAT AACCAGAATG
28150 28160 28170 28180 28190 28200 28210
>< HinP1I >< StyI
>< HaeII
> < PalI >< Hin6I >< EcoT14I
> < HaeIII >< HhaI>< Eco130I
>< BspWI >< BssT1I
> < BsuRI >< Bsp143II
>< HgaI> < BshI >< CfoI>< BsaJI >< HgaI
GAGGACGCAA TGGGGCAAGG CCAAAACAGC GCCGACCCCA AGGTTTACCC AATAATACTG CGTCTTGGTT
28220 28230 28240 28250 28260 28270 28280
>< TthHB8I
> < ScrFI
>< PalI
>< PaeR7I
>< NspIII
> < MvaI
>< HaeIII
>< EcoRII
>< Eco88I
>< XhoI > < Ecl136I
>< DsaV
>< BsuRI
>< SlaI > < BstOI
>< MnlI>< TaqI> < BstNI
>< CcrI > < BsiLI
>< HinfI >< BshI
>< TfiI>< BcoI>< BsaJI
>< MnlI >< DdeI >< AvaI > < ApyI
>< AluI >< DdeI > < NlaIII >< BfrI >< Ama87I >< MnlI
CACAGCTCTC ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC AATCAACACC
28290 28300 28310 28320 28330 28340 28350
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< Eco47I
>< Cfr13I
>< BsiZI
>< Bme18I > < Ksp632I
>< AvaII > < Eam1104I
>< AsuI > < EarI > < AluI>< MboII >< MaeIII
AATAGTGGTC CAGATGACCA AATTGGCTAC TACCGAAGAG CTACCCGACG AGTTCGTGGT GGTGACGGCA
28360 28370 28380 28390 28400 28410 28420
>< SstI
>< SduI
>< SacI
>< NspII
>< HgiAI
>< EspI
>< Eco24I >< Sau96I
>< Ecl136II >< StyI >< PalI
>< DdeI >< RmaI >< NspIV
>< CelII >< MaeI >< HaeIII
>< Bsp1286I >< EcoT14I >< Cfr13I
>< Bpu1102I >< Eco130I >< BsuRI
>< BmyI >< BssT1I > < BsrI
>< BanII >< RsaI >< BsaJI >< BsiZI

FIGURE 13.66

```
          >< Alw21I     >< Csp6I     >< BlnI     >< BshI>< HindIII
  >< HphI  >< AluI          >< AfaI     >< AvrII   >< AsuI   >< AluI
AAATGAAAGA GCTCAGCCCC AGATGGTACT TCTATTACCT AGGAACTGGC CCAGAAGCTT CACTTCCCTA
    28430      28440      28450      28460      28470      28480      28490

  >< HinP1I
  >< Hin6I
    >< HhaI
     >< HaeII
    >< CfoI                  > < MnlI       >< NlaIV
     >< Bsp143II            >< SfaNI  >< DdeI >< BscBI
CGGCGCTAAC AAAGAAGGCA TCGTATGGGT TGCAACTGAG GGAGCCTTGA ATACACCCAA AGACCACATT
    28500      28510      28520      28530      28540      28550      28560

  >< NlaIV
><  Eco64I
  >< BscBI
><  BanI
     >< AciI
><  AccB1I   >< BbvI       >< Fnu4HI                          >< MnlI
GGCACCCGCA ATCCTAATAA CAATGCTGCC ACCGTGCTAC AACTTCCTCA AGGAACAACA TTGCCAAAAG
    28570      28580      28590      28600      28610      28620      28630

                                                                  >< ThaI
                                                               >< MnlI
                                                            >< MaeII >< MvnI
                                                     >< MnlI      BstUI ><
                                 >< Fnu4HI          >< Ksp632I     Bsp50I ><
                               >< BspWI             >< EarI       >< BsaAI>< AciI
  >< MnlI    >< MnlI     >< AciI>< MboII          >< Eam1104I     AccII ><
GCTTCTACGC AGAGGGAAGC AGAGGCGGCA GTCAAGCCTC TTCTCGCTCC TCATCACGTA GTCGCGGTAA
    28640      28650      28660      28670      28680      28690      28700

                 >< ScrFI
                 >< MvaI
                >< EcoRII                        >< TthHB8I
                  >< Ecl136I                           >< RmaI
                >< DsaV>< Fnu4HI                     >< NheI
                  >< BstOI                          >< MnlI
                  >< BstNI                           >< MaeI
                  >< BsiLI                       > < BspWI
                  >< ApyI          >< BbvI      >< TaqI    >< AciI
TTCAAGAAAT TCAACTCCTG GCAGCAGTAG GGGAAATTCT CCTGCTCGAA TGGCTAGCGG AGGTGGTGAA
    28710      28720      28730      28740      28750      28760      28770

       > < ThaI
       > < MvnI
    >< HphI   >< MnlI
       > < HinP1I
       > < Hin6I
          >< HhaI
       > < BstUI     >< RmaI                                    PalI ><
       > < Bsp50I    >< MaeI                                   HaeIII ><
  >< BbvI >< CfoI>< Fnu4HI                                     BsuRI ><
       > < AccII>< BspWI            >< AluI                     BshI ><
ACTGCCCTCG CGCTATTGCT GCTAGACAGA TTGAACCAGC TTGAGAGCAA AGTTTCTGGT AAAGGCCAAC
    28780      28790      28800      28810      28820      28830      28840

                                                                 RsaI ><
      > < PalI>< MaeIII                                           >< MnlI
      > < HaeIII                  >< Fnu4HI                    MaeII ><
      > < BsuRI     >< DdeI         >< DdeI                      Csp6I ><
```

FIGURE 13.67

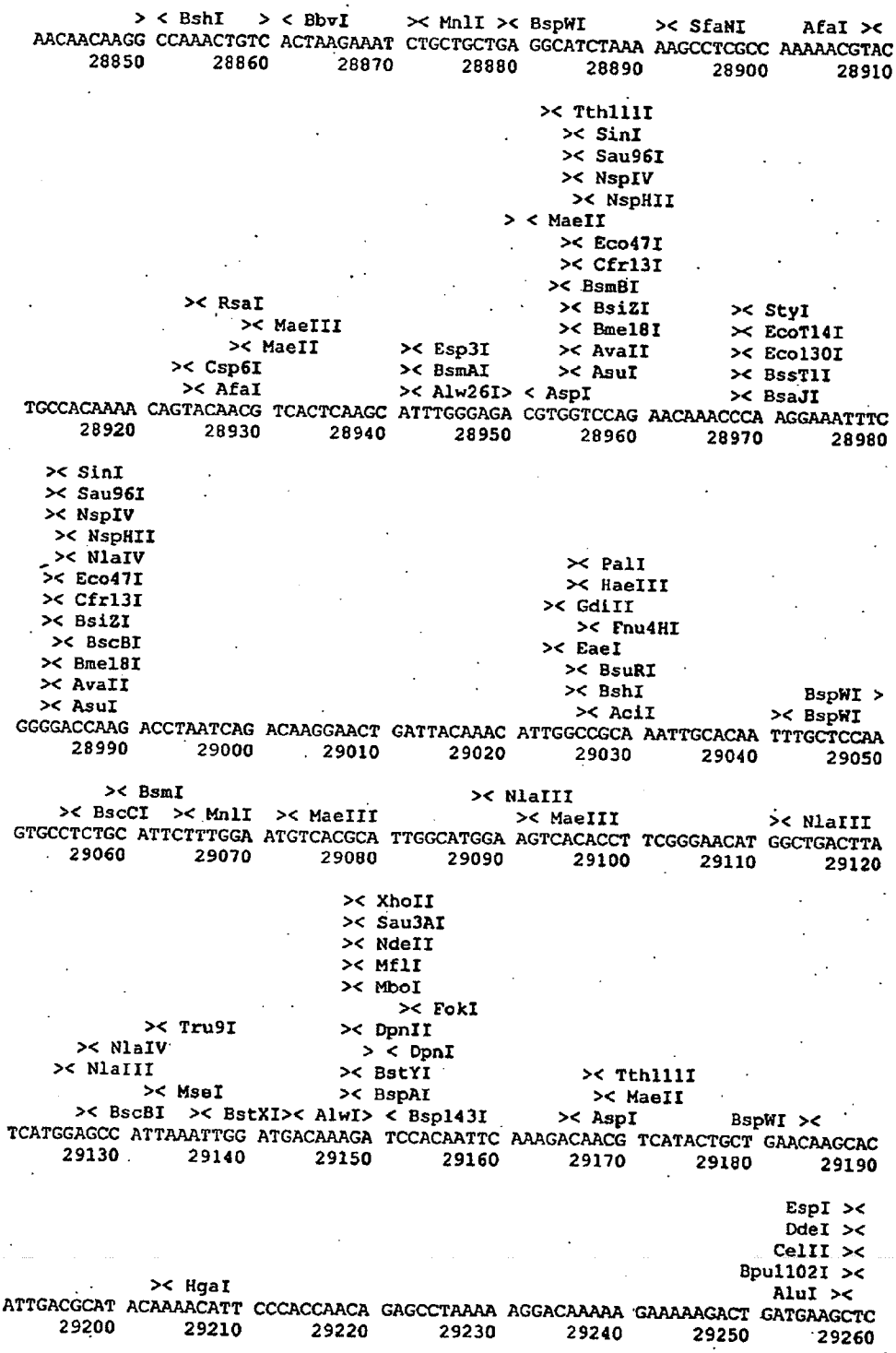

> < BshI > < BbvI >< MnlI >< BspWI >< SfaNI AfaI ><
AACAACAAGG CCAAACTGTC ACTAAGAAAT CTGCTGCTGA GGCATCTAAA AAGCCTCGCC AAAAACGTAC
28850 28860 28870 28880 28890 28900 28910
>< TthlllI
>< SinI
>< Sau96I
>< NspIV
>< NspHII
> < MaeII
>< Eco47I
>< Cfr13I
>< BsmBI
>< RsaI
>< MaeIII
>< MaeII
>< Esp3I
>< BsmAI
>< Alw26I
>< BsiZI
>< Bme18I
>< AvaII
>< AsuI
> < AspI
>< StyI
>< EcoT14I
>< Eco130I
>< BssT1I
>< BsaJI
>< Csp6I
>< AfaI
TGCCACAAAA CAGTACAACG TCACTCAAGC ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATTTC
28920 28930 28940 28950 28960 28970 28980
>< SinI
>< Sau96I
>< NspIV
>< NspHII
>< NlaIV
>< Eco47I
>< Cfr13I
>< BsiZI
>< BscBI
>< Bme18I
>< AvaII
>< AsuI
>< PalI
>< HaeIII
>< GdiII
>< Fnu4HI
>< EaeI
>< BsuRI
>< BshI
>< AciI
BspWI >
>< BspWI
GGGGACCAAG ACCTAATCAG ACAAGGAACT GATTACAAAC ATTGGCCGCA AATTGCACAA TTTGCTCCAA
28990 29000 29010 29020 29030 29040 29050
>< BsmI
>< BscCI >< MnlI >< MaeIII
>< NlaIII
>< MaeIII
>< NlaIII
GTGCCTCTGC ATTCTTTGGA ATGTCACGCA TTGGCATGGA AGTCACACCT TCGGGAACAT GGCTGACTTA
29060 29070 29080 29090 29100 29110 29120
>< XhoII
>< Sau3AI
>< NdeII
>< MflI
>< MboI
>< FokI
>< Tru9I
>< DpnII
>< NlaIV
> < DpnI
>< NlaIII
>< BstYI
>< TthlllI
>< BspAI
>< MaeII
>< MseI
>< BscBI >< BstXI>< AlwI> < Bsp143I >< AspI BspWI ><
TCATGGAGCC ATTAAATTGG ATGACAAAGA TCCACAATTC AAAGACAACG TCATACTGCT GAACAAGCAC
29130 29140 29150 29160 29170 29180 29190
EspI ><
DdeI ><
CelII ><
Bpu1102I ><
AluI ><
>< HgaI
ATTGACGCAT ACAAAACATT CCCACCAACA GAGCCTAAAA AGGACAAAAA GAAAAAGACT GATGAAGCTC
29200 29210 29220 29230 29240 29250 29260

FIGURE 13.68

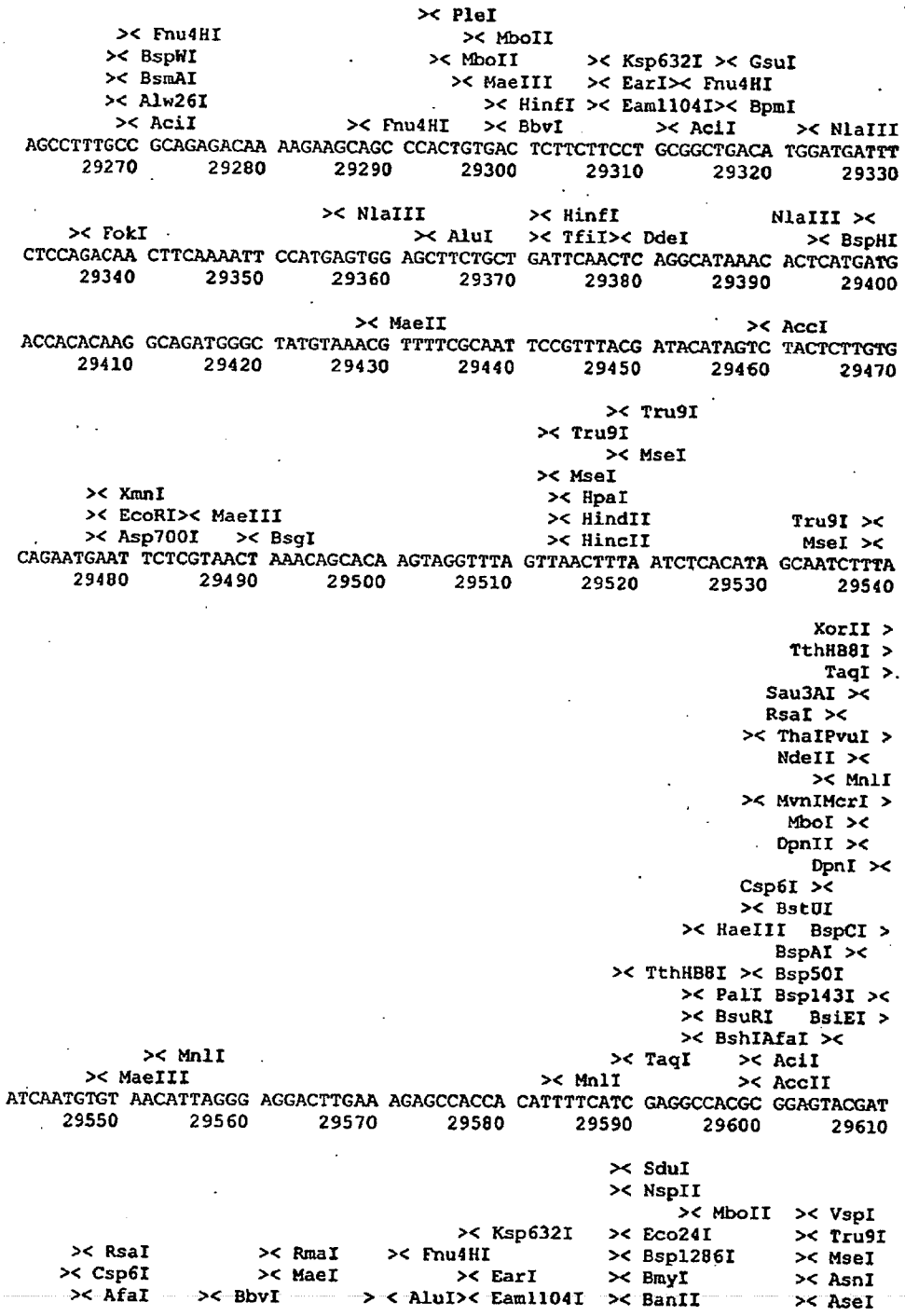

>< PleI
>< Fnu4HI
>< MboII
>< BspWI
>< MboII
>< Ksp632I >< GsuI
>< BsmAI
>< MaeIII
>< EarI>< Fnu4HI
>< Alw26I
>< HinfI >< Eam1104I>< BpmI
>< AciI
>< Fnu4HI
>< BbvI
>< AciI
>< NlaIII
AGCCTTTGCC GCAGAGACAA AAGAAGCAGC CCACTGTGAC TCTTCTTCCT GCGGCTGACA TGGATGATTT
29270 29280 29290 29300 29310 29320 29330
>< NlaIII
>< HinfI
NlaIII ><
>< FokI
>< AluI
>< TfiI>< DdeI
>< BspHI
CTCCAGACAA CTTCAAAATT CCATGAGTGG AGCTTCTGCT GATTCAACTC AGGCATAAAC ACTCATGATG
29340 29350 29360 29370 29380 29390 29400
>< MaeII
>< AccI
ACCACACAAG GCAGATGGGC TATGTAAACG TTTTCGCAAT TCCGTTTACG ATACATAGTC TACTCTTGTG
29410 29420 29430 29440 29450 29460 29470
>< Tru9I
>< Tru9I
>< MseI
>< MseI
>< XmnI
>< HpaI
>< EcoRI>< MaeIII
>< HindII
Tru9I ><
>< Asp700I >< BsgI
>< HincII
MseI ><
CAGAATGAAT TCTCGTAACT AAACAGCACA AGTAGGTTTA GTTAACTTTA ATCTCACATA GCAATCTTTA
29480 29490 29500 29510 29520 29530 29540
XorII >
TthHB8I >
TaqI >
Sau3AI ><
RsaI ><
>< ThaIPvuI >
NdeII ><
>< MnlI
>< MvnIMcrI >
MboI ><
DpnII ><
DpnI ><
Csp6I ><
>< BstUI
>< HaeIII BspCI >
BspAI ><
>< TthHB8I >< Bsp50I
>< PalI Bsp143I ><
>< BsuRI BsiEI >
>< BshIAfaI ><
>< MnlI
>< TaqI >< AciI
>< MaeIII
>< MnlI
>< AccII
ATCAATGTGT AACATTAGGG AGGACTTGAA AGAGCCACCA CATTTTCATC GAGGCCACGC GGAGTACGAT
29550 29560 29570 29580 29590 29600 29610
>< SduI
>< NspII
>< MboII
>< VspI
>< Ksp632I
>< Eco24I
>< Tru9I
>< RsaI
>< RmaI
>< Fnu4HI
>< Bsp1286I
>< MseI
>< Csp6I
>< MaeI
>< EarI
>< BmyI
>< AsnI
>< AfaI
>< BbvI
> < AluI>< Eam1104I
>< BanII
>< AseI

FIGURE 13.69

```
CGAGGGTACA GTGAATAATG CTAGGGAGAG CTGCCTATAT GGAAGAGCCC TAATGTGTAA AATTAATTTT
    29620      29630      29640      29650      29660      29670      29680

                        >< Tru9I   >< DdeI
                        >< MseI    >< BfrI
                    >< NlaIII    > < AluI
AGTAGTGCTA TCCCCATGTG ATTTTAATAG CTTCTTAGGA GAATGACAAA AAAAAAAAAA AAAAAA
    29690      29700      29710      29720      29730      29740
```

FIGURE 13. 70

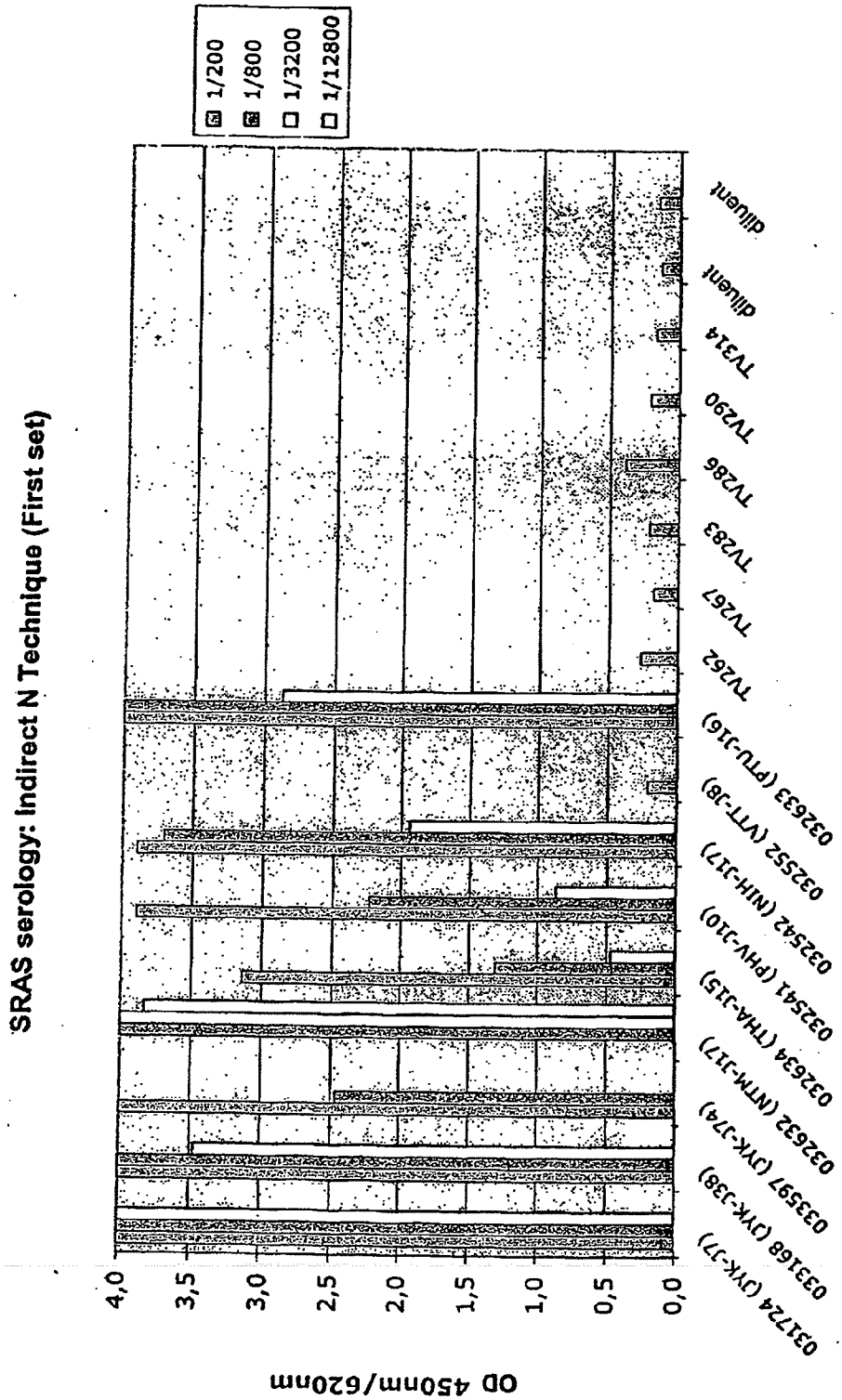

SRAS serology: Indirect N Technique (First set)
1/200
1/800
1/3200
1/12800
OD 450nm/620nm
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
031724 (JYK-377)
033168 (JYK-338)
033597 (JYK-374)
032632 (NTM-317)
032634 (THA-315)
032541 (PHV-310)
032542 (NIH-317)
032552 (VTT-38)
032633 (PTU-316)
TV262
TV267
TV283
TV286
TV290
TV314
diluent
diluent
diluent

FIGURE 14

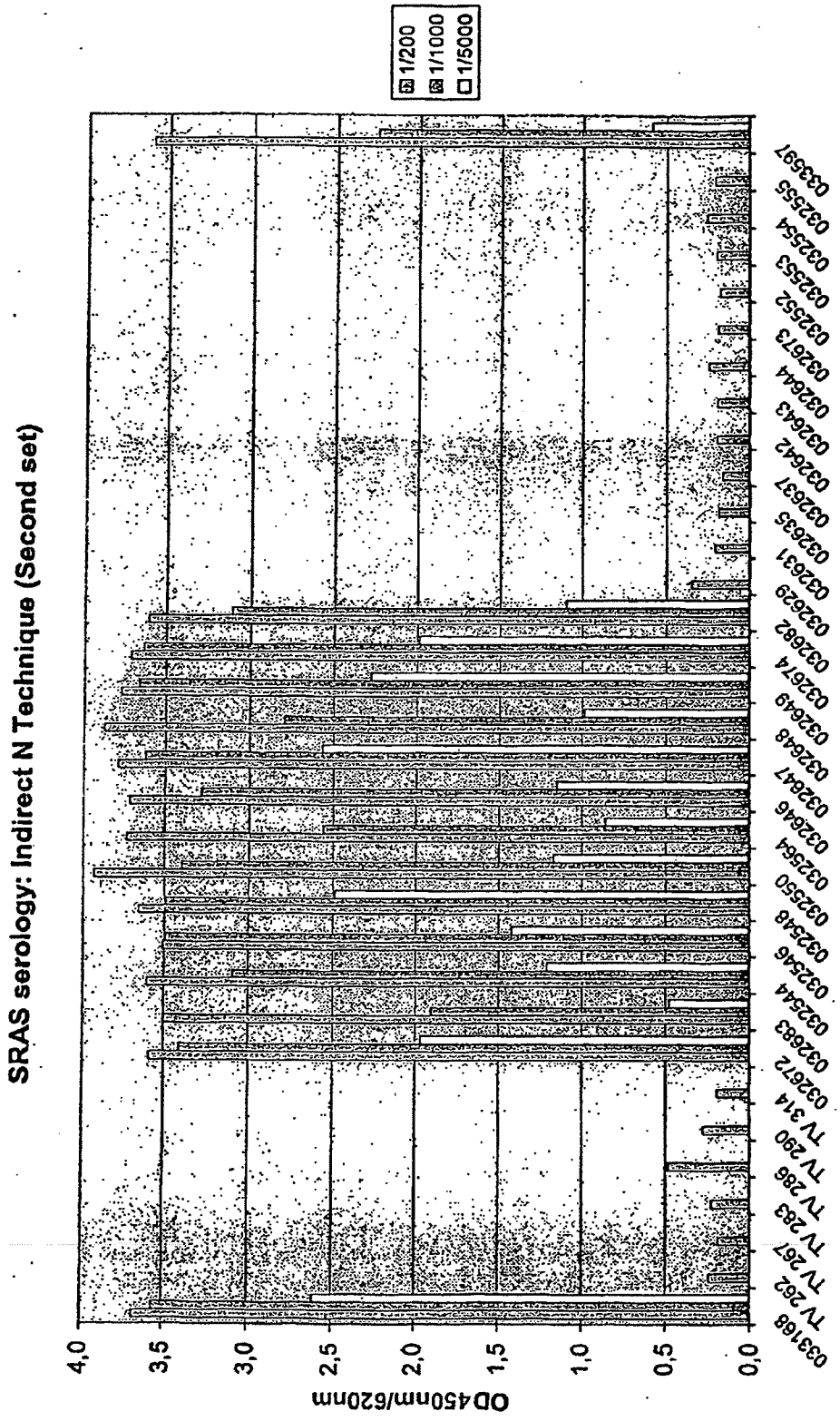

SRAS serology: Indirect N Technique (Second set)
1/200
1/1000
1/5000
OD450nm/620nm
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Number of specimens
TV 168
TV 262
TV 267
TV 283
TV 286
TV 290
TV 314
032672
032683
032544
032546
032548
032550
032564
032646
032647
032648
032649
032674
032682
032629
032631
032635
032637
032642
032643
032644
032673
032652
032653
032654
032655
032697

FIGURE 15

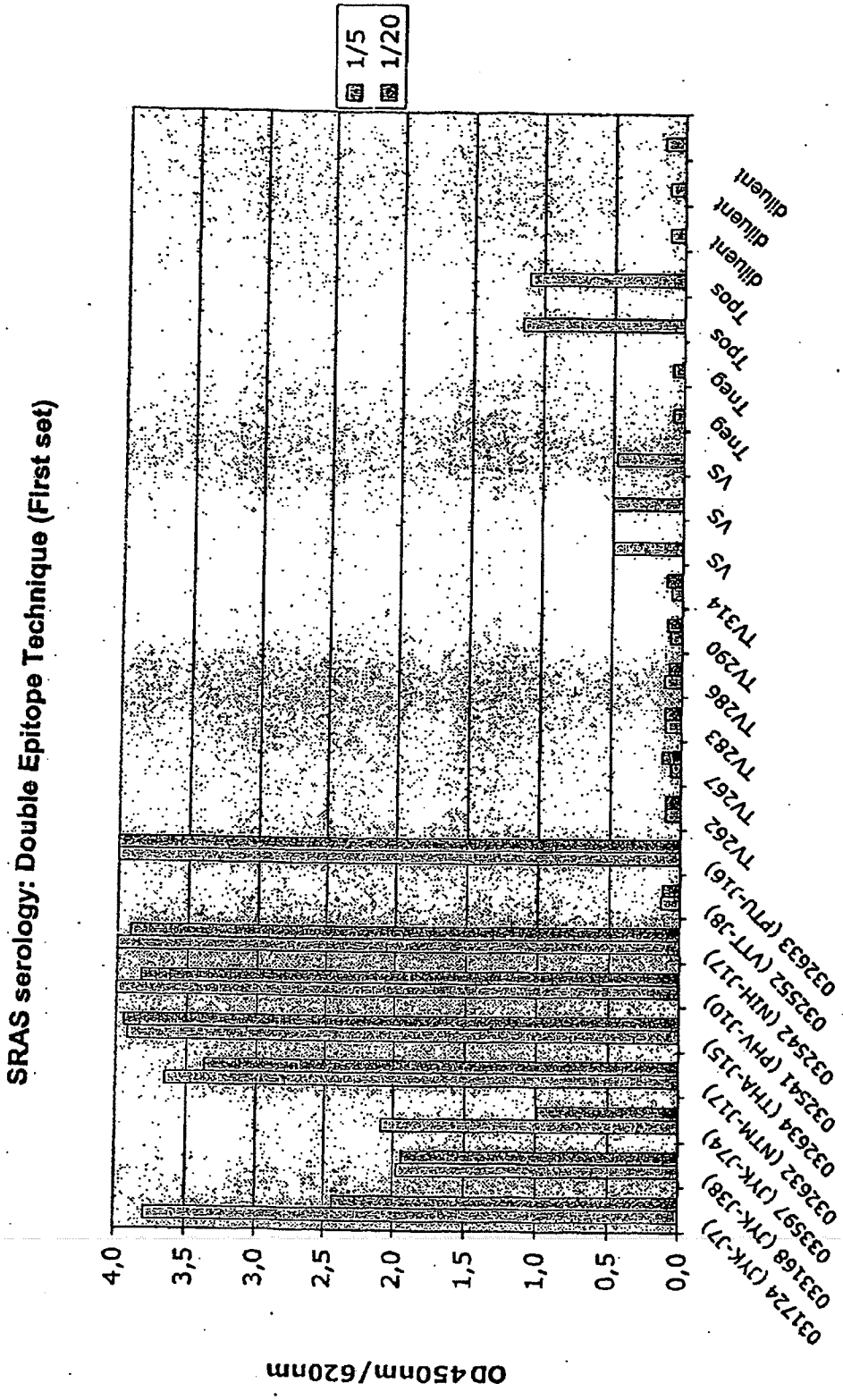

SRAS serology: Double Epitope Technique (First set)
1/5
1/20
OD450nm/620nm
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
031724 (JYK-J7)
033168 (JYK-J38)
033597 (JYK-J74)
032632 (NTM-J17)
032634 (TMA-J15)
032541 (PHV-J10)
032542 (NIH-J17)
032552 (VTT-J8)
032633 (PTU-J16)
TV262
TV267
TV283
TV286
TV290
TV314
VS
VS
VS
Tneg
Tneg
Tpos
Tpos
diluent
diluent
diluent

FIGURE 16

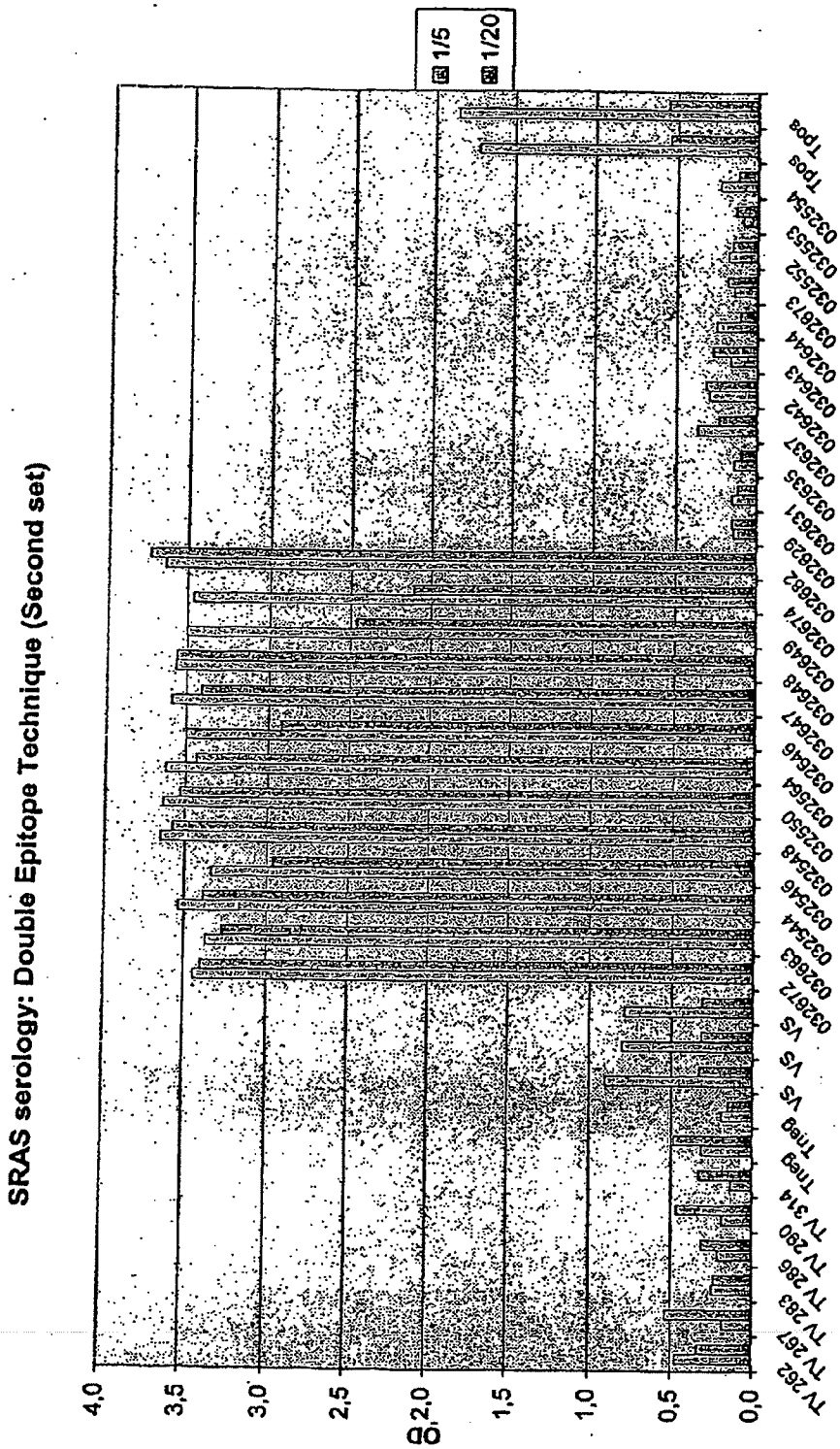

SRAS serology: Double Epitope Technique (Second set)
1/5
1/20
OD
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
TV 262
TV 267
TV 283
TV 286
TV 290
TV 314
Treg VS
VS
032672
032663
032654
032646
032648
032650
032654
032646
032647
032648
032649
032674
032682
032829
032631
032635
032637
032642
032643
032644
032673
032652
032653
032654
Tpos
Tpos
Number of specimens

FIGURE 17

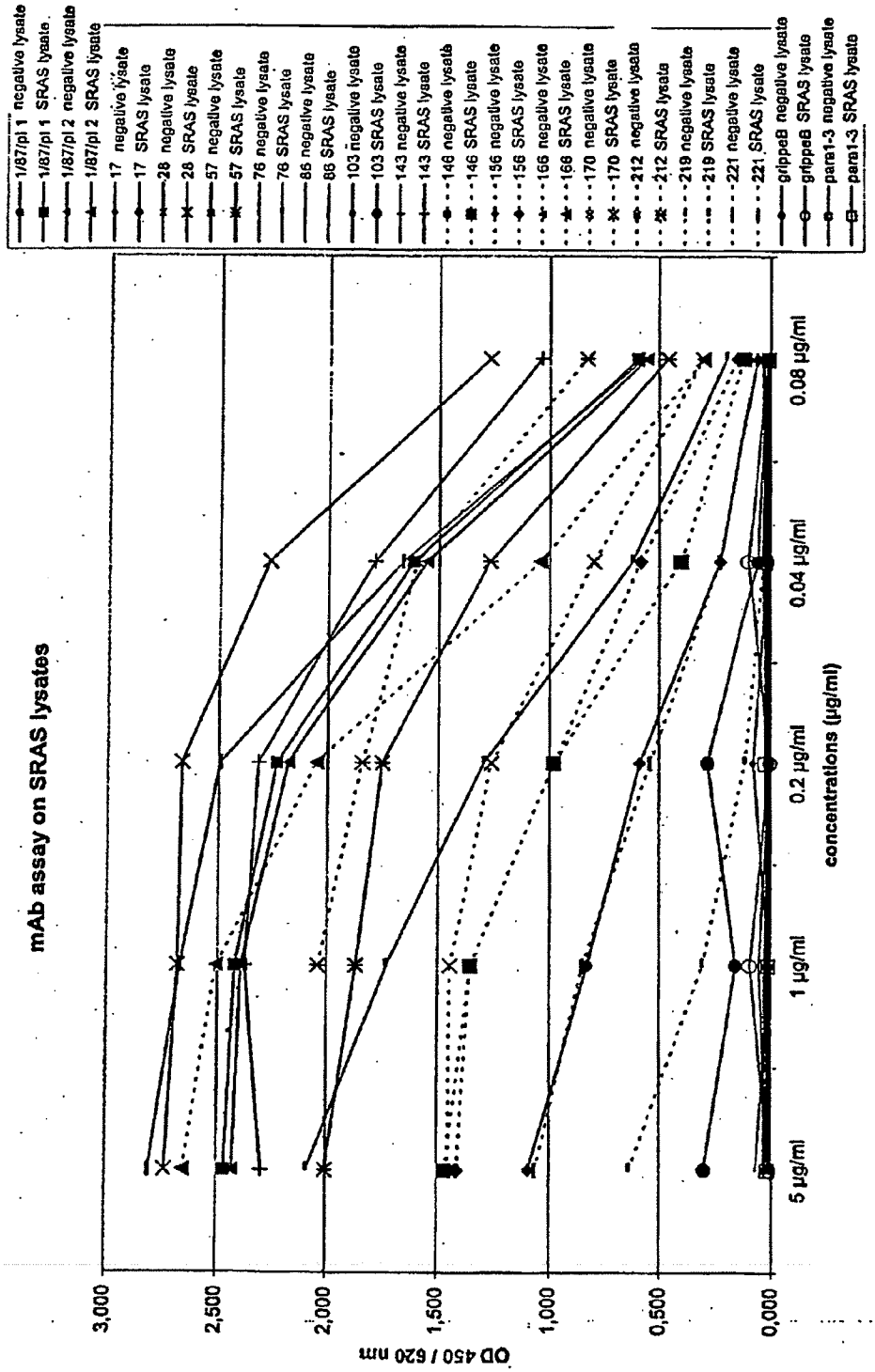

mAb assay on SRAS lysates
OD 450 / 620 nm
3,000
2,500
2,000
1,500
1,000
0,500
0,000
5 µg/ml
1 µg/ml
0.2 µg/ml
0.04 µg/ml
0.08 µg/ml
concentrations (µg/ml)
1/87/pl 1 negative lysate
1/87/pl 1 SRAS lysate
1/87/pl 2 negative lysate
1/87/pl 2 SRAS lysate
17 negative lysate
17 SRAS lysate
28 negative lysate
28 SRAS lysate
57 negative lysate
57 SRAS lysate
76 negative lysate
76 SRAS lysate
86 negative lysate
86 SRAS lysate
103 negative lysate
103 SRAS lysate
143 negative lysate
143 SRAS lysate
146 negative lysate
146 SRAS lysate
156 negative lysate
158 SRAS lysate
166 negative lysate
168 SRAS lysate
170 negative lysate
170 SRAS lysate
212 negative lysate
212 SRAS lysate
219 negative lysate
219 SRAS lysate
221 negative lysate
221 SRAS lysate
grippeB negative lysate
grippeB SRAS lysate
para1-3 negative lysate
para1-3 SRAS lysate

FIGURE 18

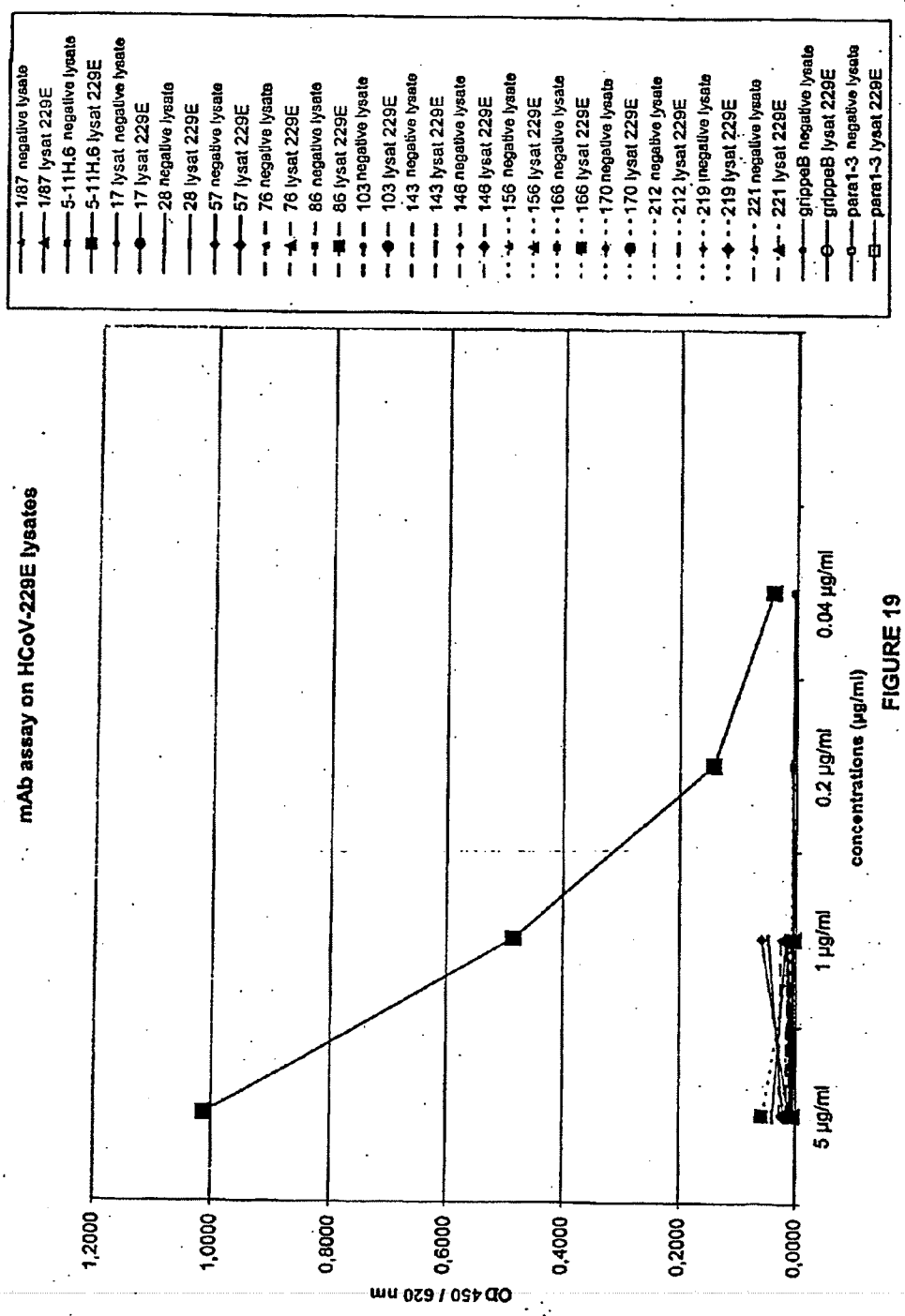

mAb assay on HCoV-229E lysates
1/87 negative lysate
1/87 lysat 229E
5-11H.6 negative lysate
5-11H.6 lysat 229E
17 lysat negative lysate
17 lysat 229E
28 negative lysate
28 lysat 229E
57 negative lysate
57 lysat 229E
76 negative lysate
76 lysat 229E
86 negative lysate
86 lysat 229E
103 negative lysate
103 lysat 229E
143 negative lysate
143 lysat 229E
146 negative lysate
146 lysat 229E
156 negative lysate
156 lysat 229E
166 negative lysate
166 lysat 229E
170 negative lysate
170 lysat 229E
212 negative lysate
212 lysat 229E
219 negative lysate
219 lysat 229E
221 negative lysate
221 lysat 228E
grippeB negative lysate
grippeB lysat 229E
para1-3 negative lysate
para1-3 lysat 229E
OD 450 / 620 nm
1,2000
1,0000
0,8000
0,6000
0,4000
0,2000
0,0000
5 µg/ml
1 µg/ml
0.2 µg/ml
0.04 µg/ml
concentrations (µg/ml)

FIGURE 19

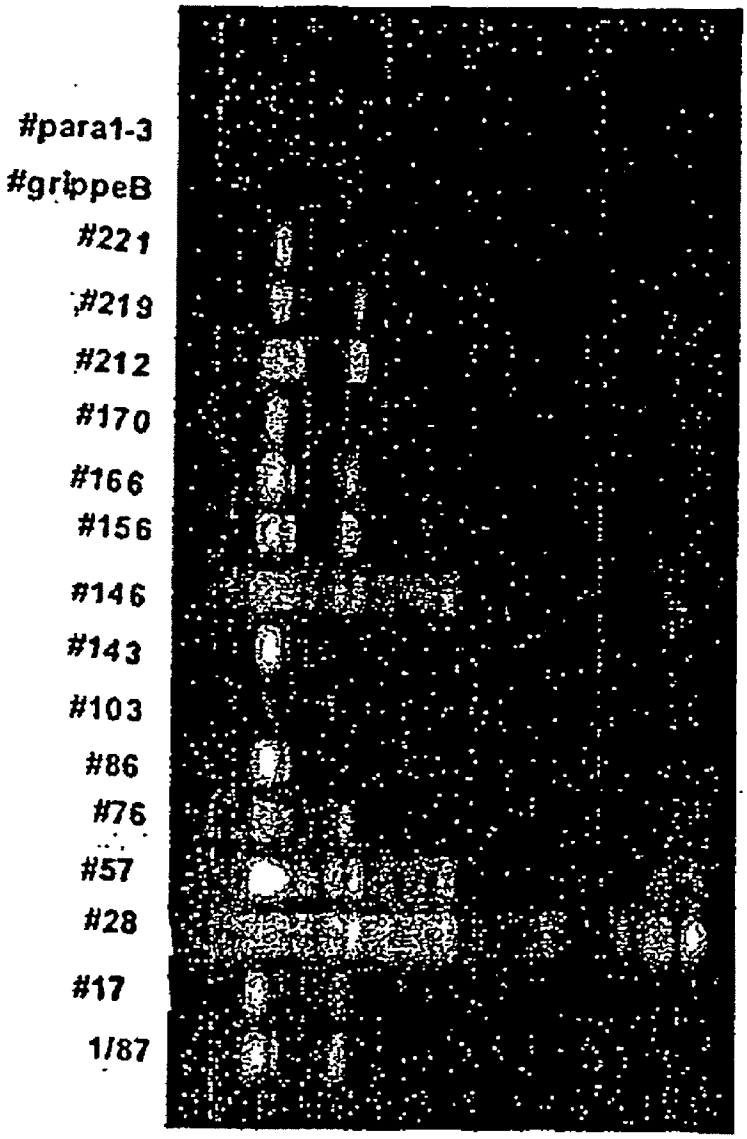

#para1-3
#grippeB
#221
#219
#212
#170
#166
#156
#146
#143
#103
#86
#76
#57
#28
#17
1/87

FIGURE 20

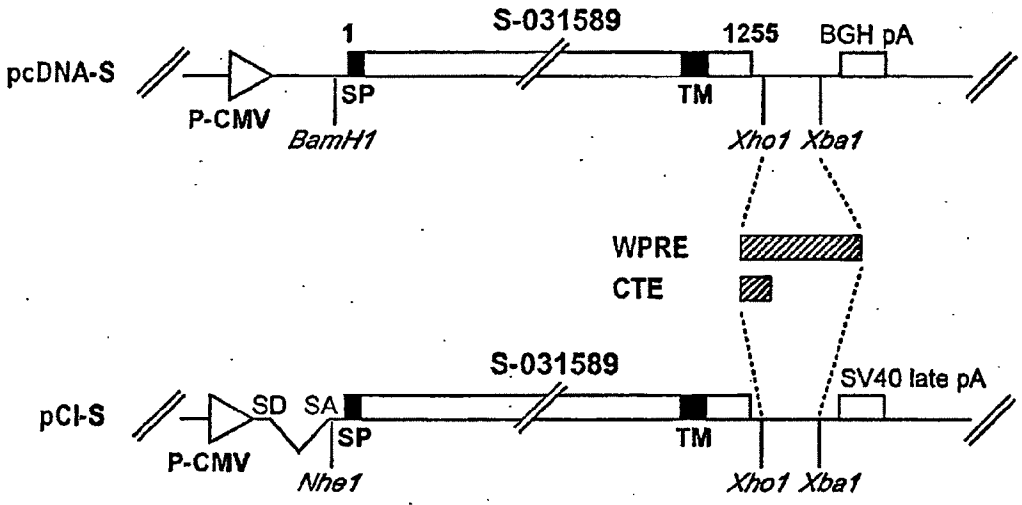

pcDNA-S
P-CMV
1
SP
BamH1
S-031589
TM
1255
BGH pA
Xho1
Xba1
WPRE
CTE
pCI-S
SD
SA
SP
Nhe1
S-031589
TM
SV40 late pA
Xho1
Xba1

FIGURE 21

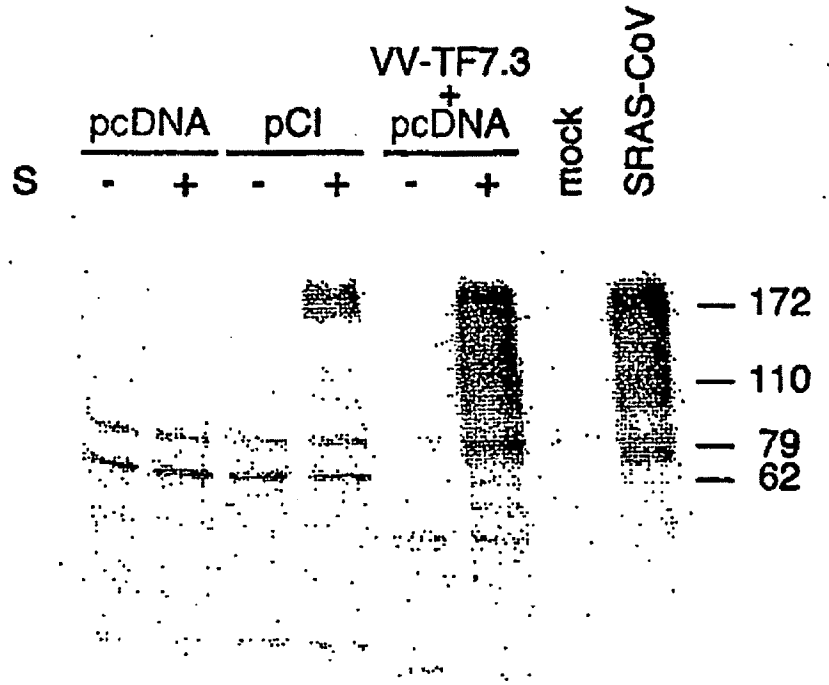
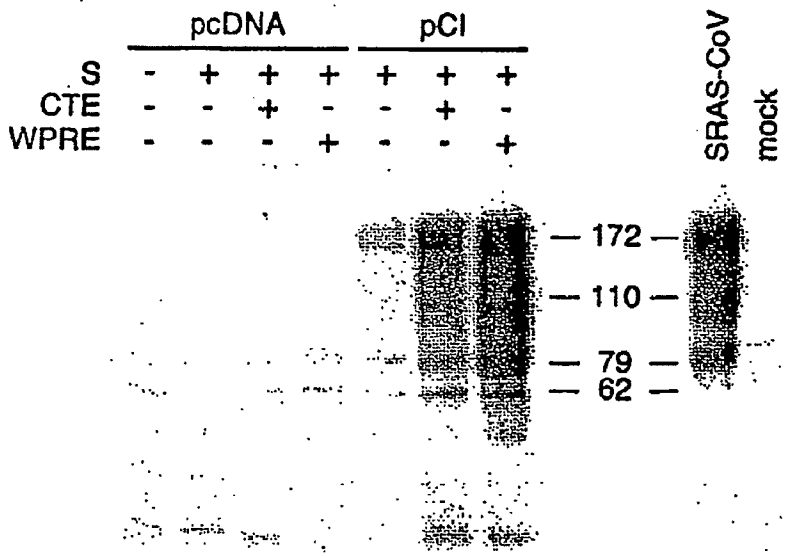


FIGURE 22

A.


pcDNA
pCI
S - + + + + + +
CTE - - + - - + -
WPRE - - - + - - +
SRAS-CoV
mock
172
110
79
62

B.

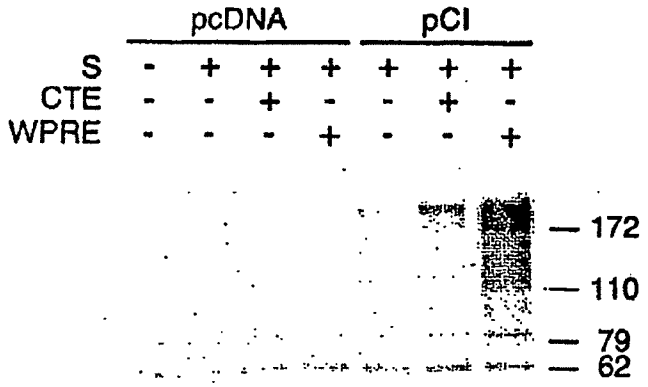

pcDNA
pCI
S - + + + + + +
CTE - - + - - + -
WPRE - - - + - - +
172
110
79
62

FIGURE 23

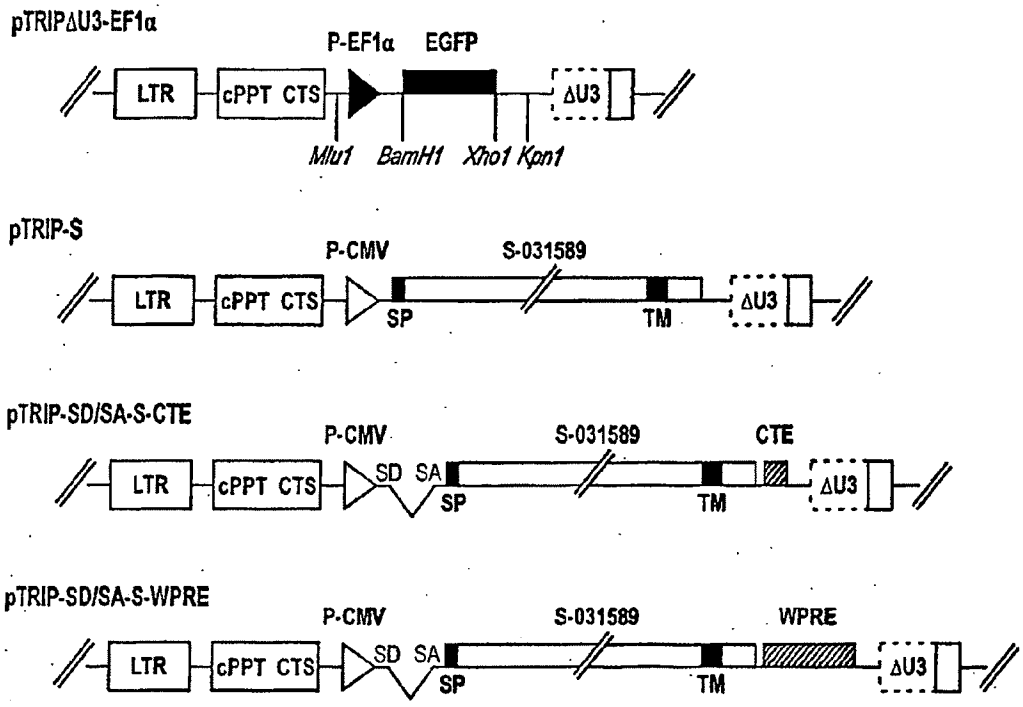

pTRIPΔU3-EF1α
P-EF1α
EGFP
LTR
cPPT CTS
ΔU3
Mlu1
BamH1
Xho1
Kpn1
pTRIP-S
P-CMV
S-031589
SP
TM
pTRIP-SD/SA-S-CTE
SD
SA
CTE
pTRIP-SD/SA-S-WPRE
WPRE

FIGURE 24

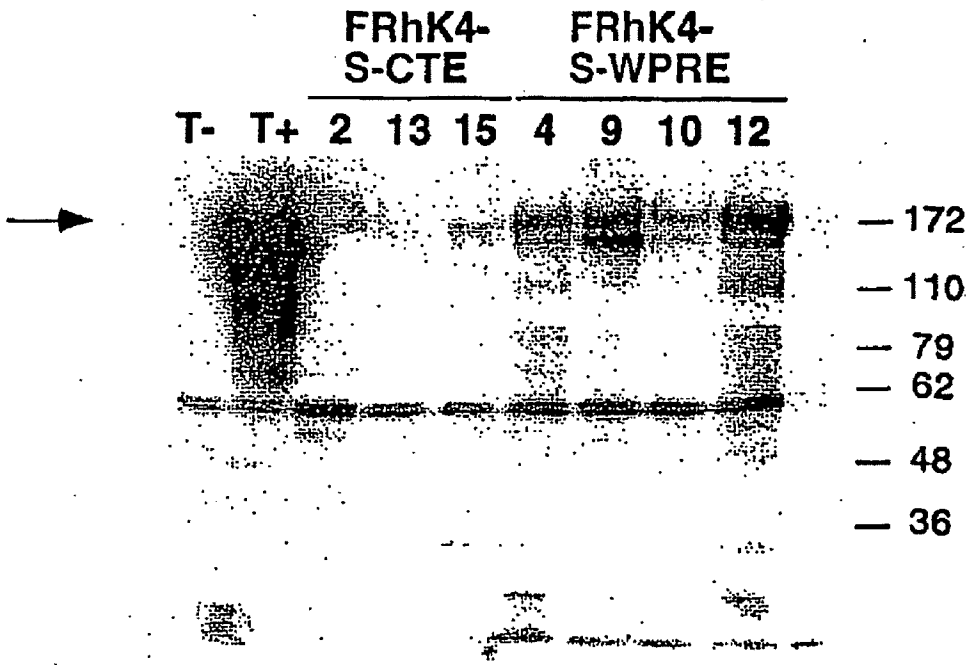


FIGURE 25

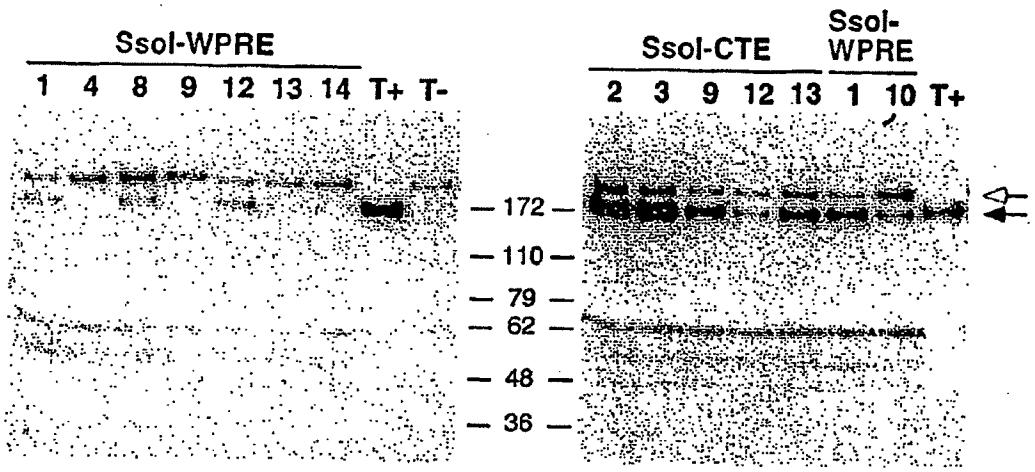
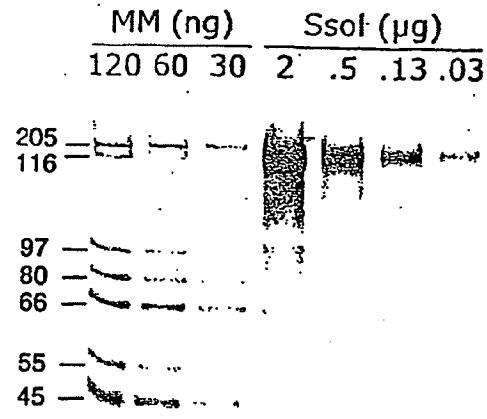
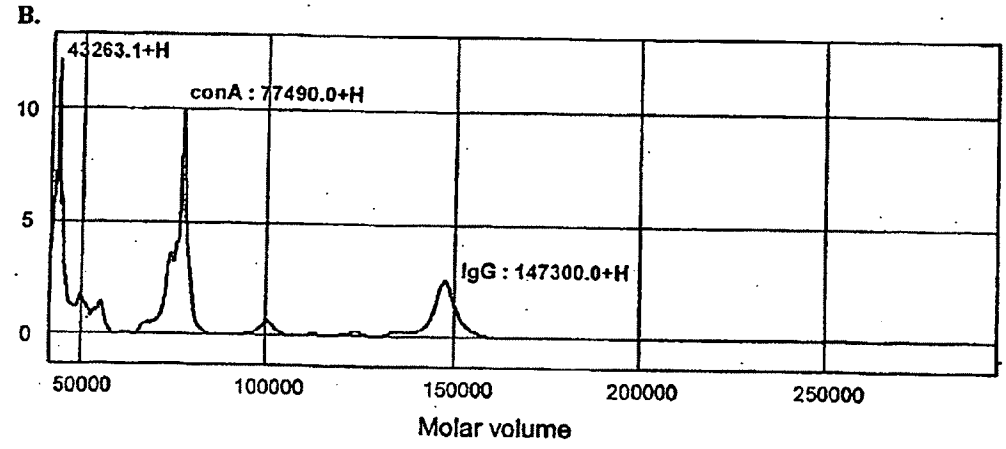

Ssol-WPRE
1 4 8 9 12 13 14 T+ T-
Ssol-CTE
Ssol-WPRE
2 3 9 12 13 1 10 T+
172
110
79
62
48
36

FIGURE 26

A.



B.



C.

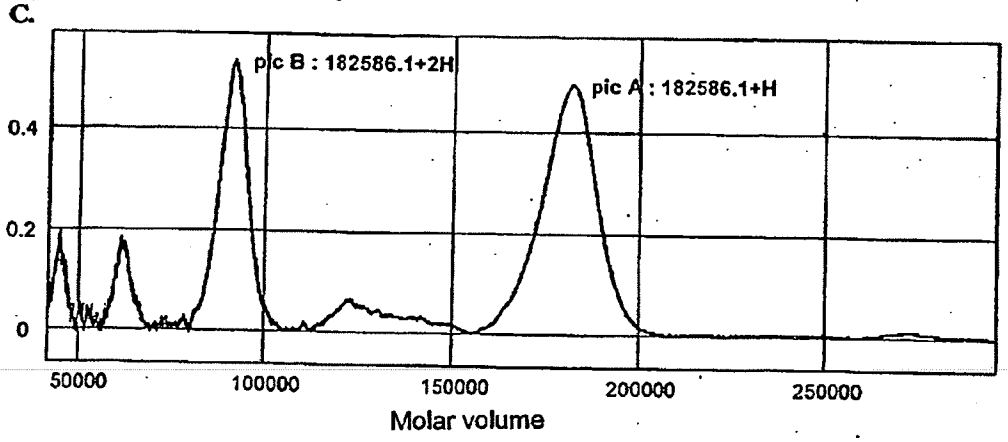


FIGURE 27 A-C

D.

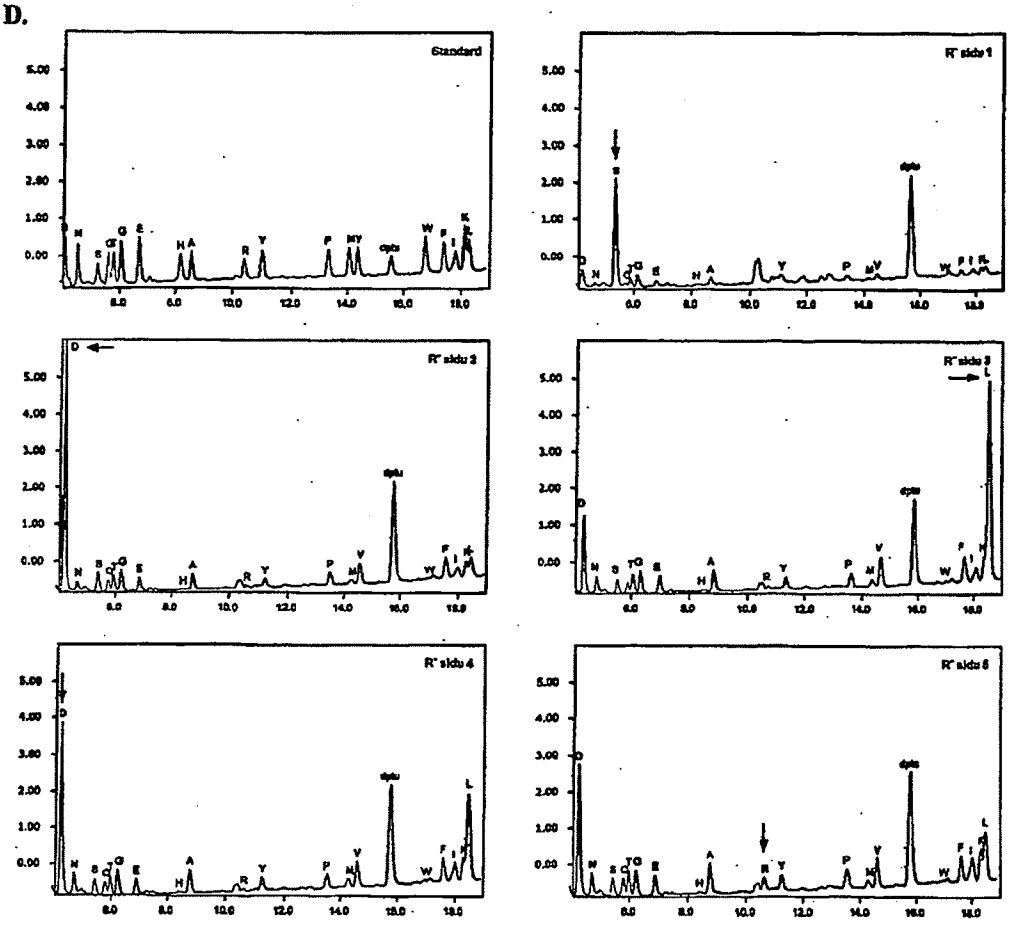
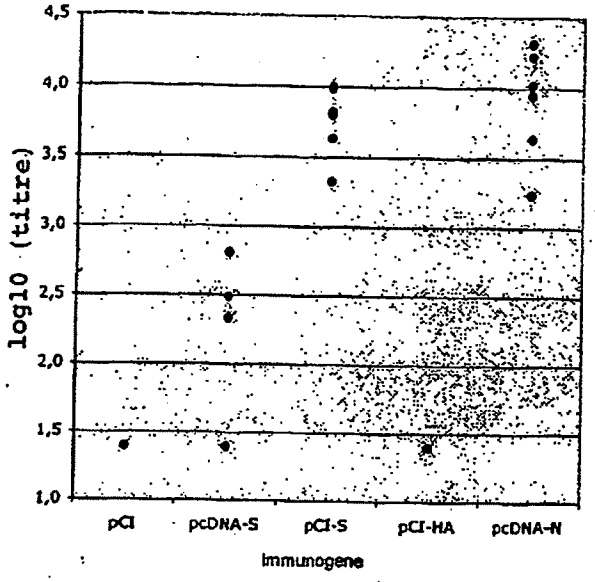


FIGURE 27 D

A.


4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
log10 (titre)
pCI
pcDNA-S
pCI-S
pCI-HA
pcDNA-N
Immunogene

B.

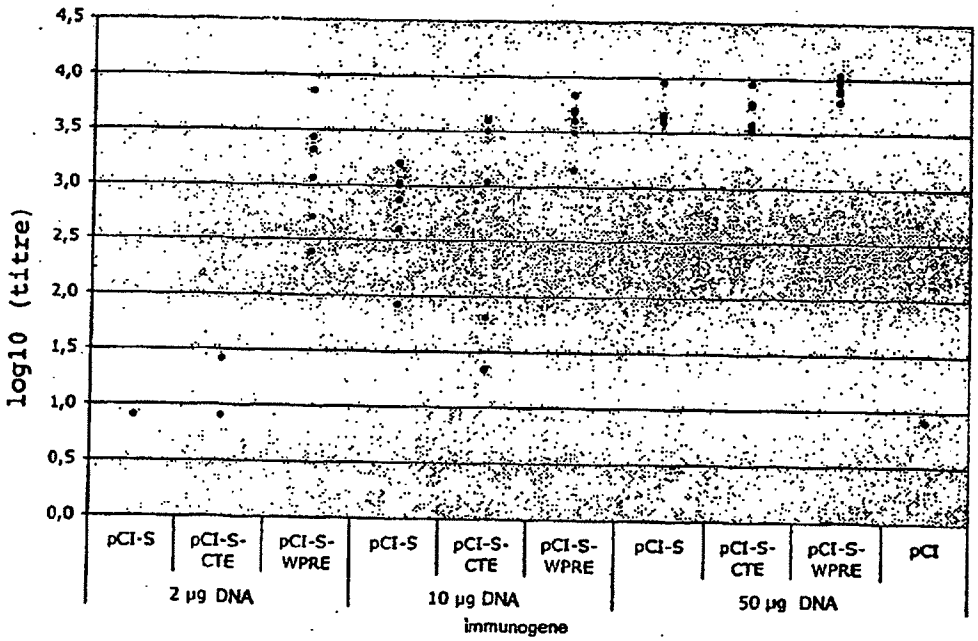
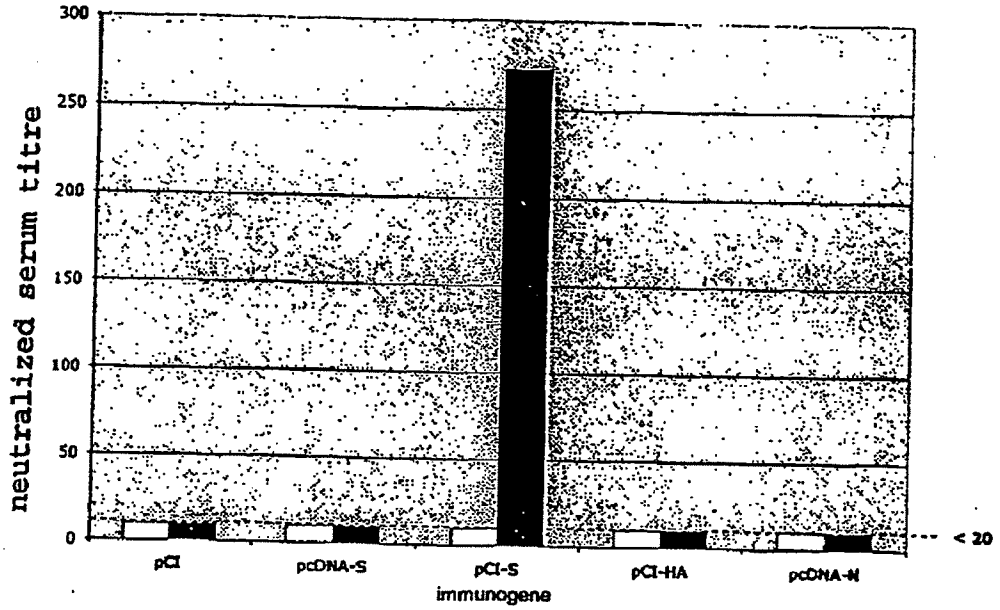

4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
log10 (titre)
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI
2 µg DNA
10 µg DNA
50 µg DNA
Immunogene

FIGURE 28

A.


neutralized serum titre
300
250
200
150
100
50
0
< 20
pCI
pcDNA-S
pCI-S
pCI-HA
pcDNA-N
immunogene

B.

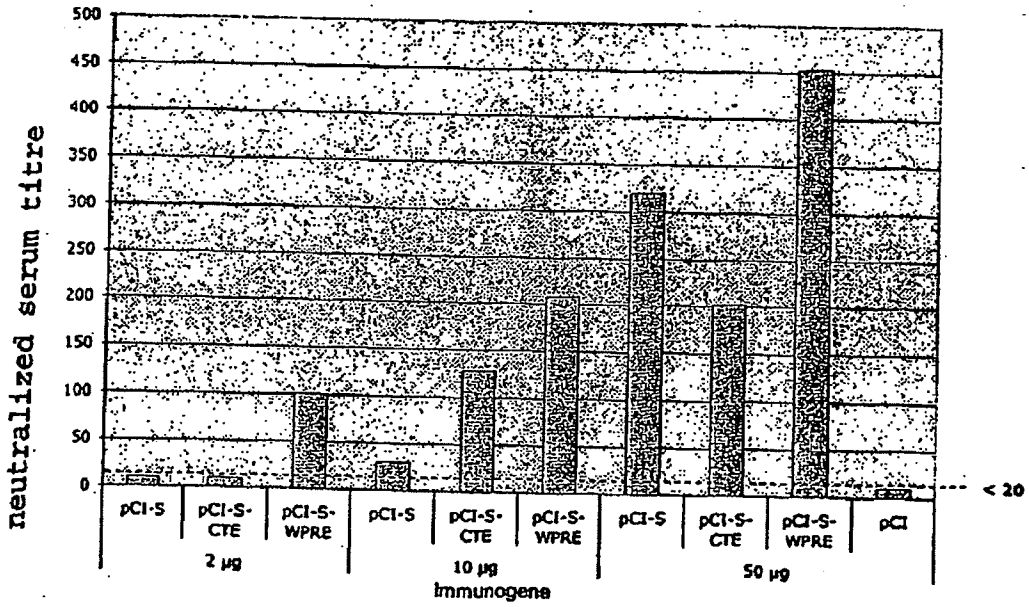

neutralized serum titre
500
450
400
350
300
250
200
150
100
50
0
< 20
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI-S
pCI-S-CTE
pCI-S-WPRE
pCI
2 µg
10 µg
50 µg
Immunogene

FIGURE 29

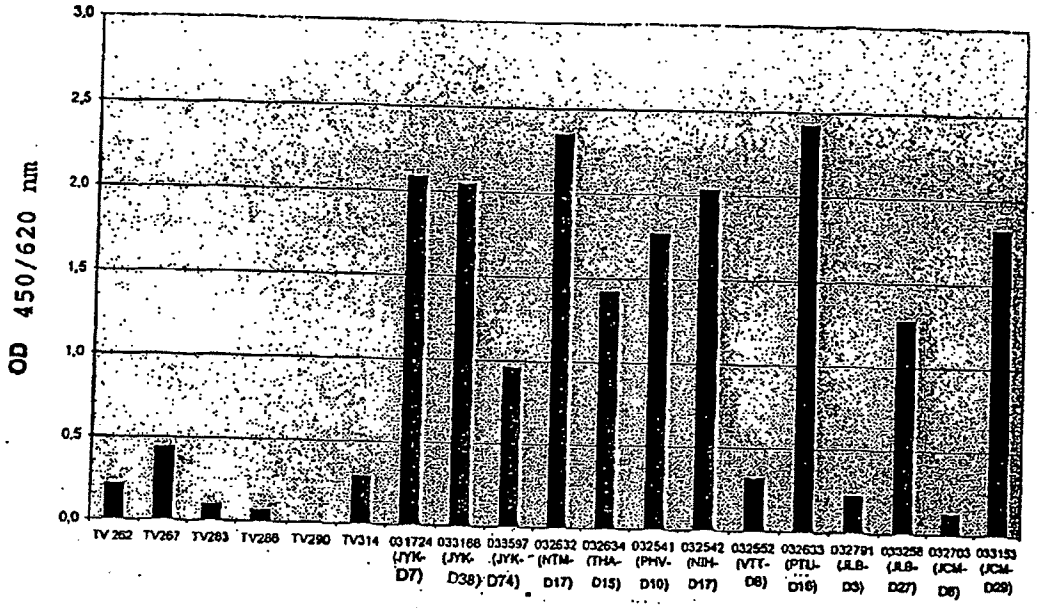

OD 450/620 nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
TV 262
TV267
TV283
TV288
TV290
TV314
031724 (JYK-D7)
033168 (JYK-D38)
033597 (JYK-D74)
032632 (NTM-D17)
032634 (THA-D15)
032541 (PHV-D10)
032542 (NIH-D17)
032552 (VTT-D8)
032633 (PTU-D16)
032791 (JLB-D3)
033258 (JLB-D27)
032703 (JCM-D6)
033153 (JCM-D29)
serums

FIGURE 30

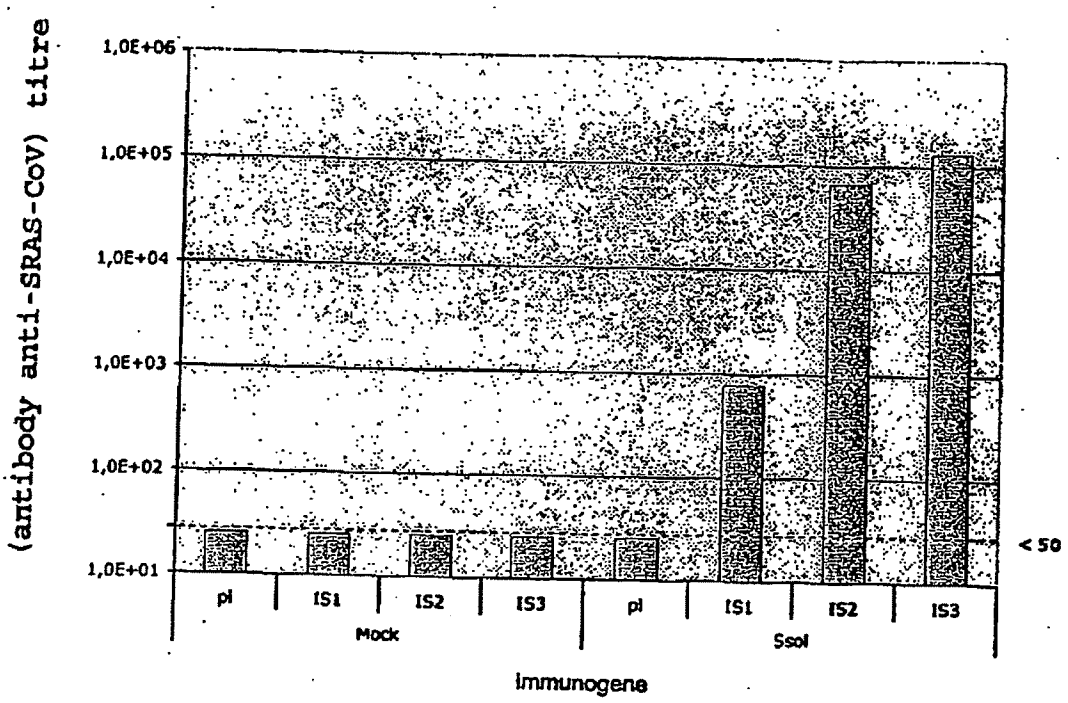

(antibody anti-SRAS-CoV) titre
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
< 50
pi
IS1
IS2
IS3
pi
IS1
IS2
IS3
Mock
Ssol
Immunogene

FIGURE 31

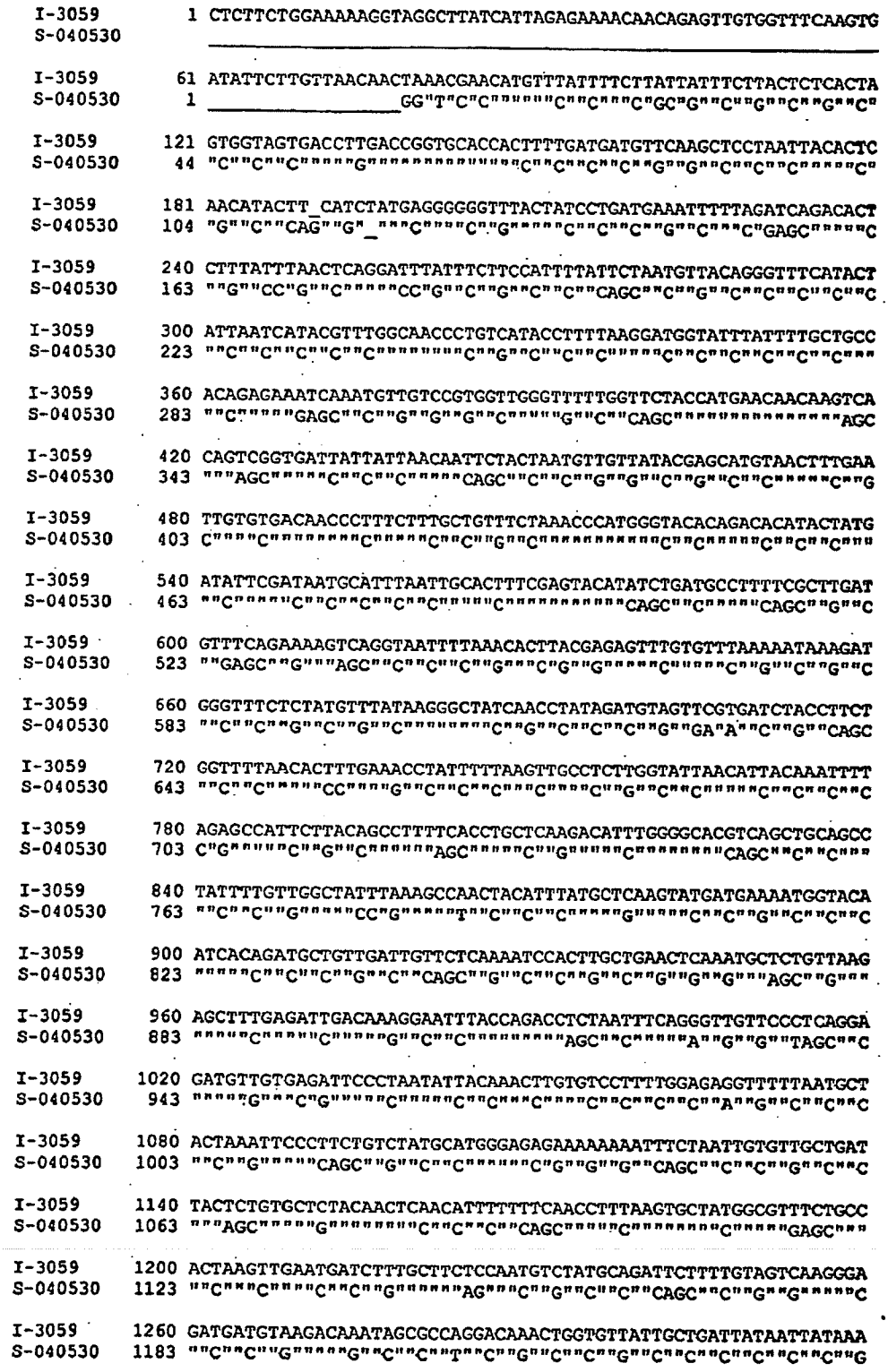

I-3059 1 CTCTTCTGGAAAAAGGTAGGCTTATCATTAGAGAAAACAACAGAGTTGTGGTTTCAAGTG
S-040530
I-3059 61 ATATTCTTGTTAACAACTAAACGAACATGTTTATTTTCTTATTATTTCTTACTCTCACTA
S-040530 1
I-3059 121 GTGGTAGTGACCTTGACCGGTGCACCACTTTTGATGATGTTCAAGCTCCTAATTACACTC
S-040530 44
I-3059 181 AACATACTT_CATCTATGAGGGGGGTTTACTATCCTGATGAAATTTTTAGATCAGACACT
S-040530 104
I-3059 240 CTTTATTTAACTCAGGATTTATTTCTTCCATTTTATTCTAATGTTACAGGGTTTCATACT
S-040530 163
I-3059 300 ATTAATCATACGTTTGGCAACCCTGTCATACCTTTTAAGGATGGTATTTATTTTGCTGCC
S-040530 223
I-3059 360 ACAGAGAAATCAAATGTTGTCCGTGGTTGGGTTTTTGGTTCTACCATGAACAACAAGTCA
S-040530 283
I-3059 420 CAGTCGGTGATTATTATTAACAATTCTACTAATGTTGTTATACGAGCATGTAACTTTGAA
S-040530 343
I-3059 480 TTGTGTGACAACCCTTTCTTTGCTGTTTCTAAACCCATGGGTACACAGACACATACTATG
S-040530 403
I-3059 540 ATATTCGATAATGCATTTAATTGCACTTTCGAGTACATATCTGATGCCTTTTCGCTTGAT
S-040530 463
I-3059 600 GTTTCAGAAAAGTCAGGTAATTTTAAACACTTACGAGAGTTTGTGTTTAAAAATAAAGAT
S-040530 523
I-3059 660 GGGTTTCTCTATGTTTATAAGGGCTATCAACCTATAGATGTAGTTCGTGATCTACCTTCT
S-040530 583
I-3059 720 GGTTTTAACACTTTGAAACCTATTTTTAAGTTGCCTCTTGGTATTAACATTACAAATTTT
S-040530 643
I-3059 780 AGAGCCATTCTTACAGCCTTTTCACCTGCTCAAGACATTTGGGGCACGTCAGCTGCAGCC
S-040530 703
I-3059 840 TATTTTGTTGGCTATTTAAAGCCAACTACATTTATGCTCAAGTATGATGAAAATGGTACA
S-040530 763
I-3059 900 ATCACAGATGCTGTTGATTGTTCTCAAAATCCACTTGCTGAACTCAAATGCTCTGTTAAG
S-040530 823
I-3059 960 AGCTTTGAGATTGACAAAGGAATTTACCAGACCTCTAATTTCAGGGTTGTTCCCTCAGGA
S-040530 883
I-3059 1020 GATGTTGTGAGATTCCCTAATATTACAAACTTGTGTCCTTTTGGAGAGGTTTTTAATGCT
S-040530 943
I-3059 1080 ACTAAATTCCCTTCTGTCTATGCATGGGAGAGAAAAAAAATTTCTAATTGTGTTGCTGAT
S-040530 1003
I-3059 1140 TACTCTGTGCTCTACAACTCAACATTTTTTTCAACCTTTAAGTGCTATGGCGTTTCTGCC
S-040530 1063
I-3059 1200 ACTAAGTTGAATGATCTTTGCTTCTCCAATGTCTATGCAGATTCTTTTGTAGTCAAGGGA
S-040530 1123
I-3059 1260 GATGATGTAAGACAAATAGCGCCAGGACAAACTGGTGTTATTGCTGATTATAATTATAAA
S-040530 1183

FIGURE 32.1

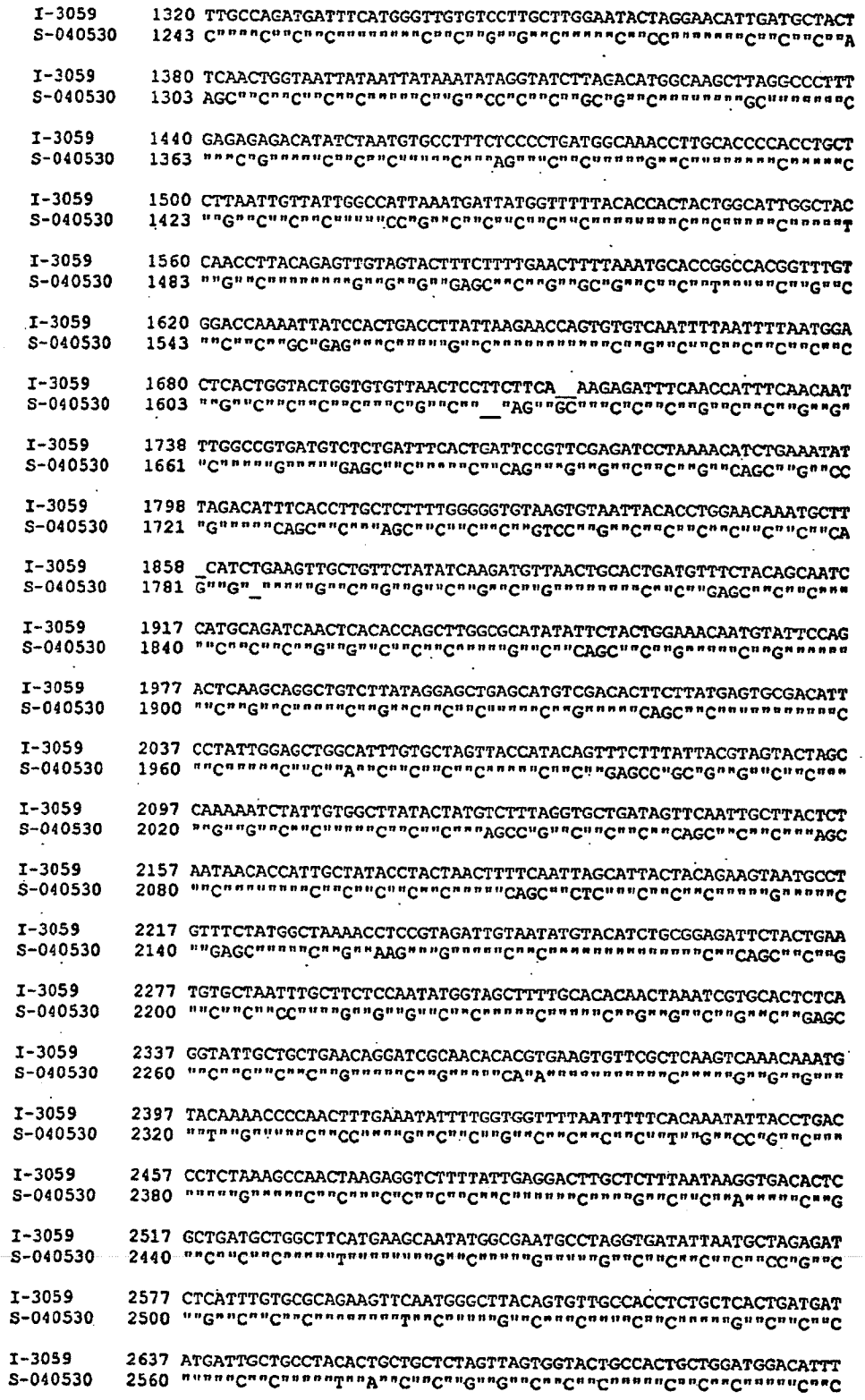

I-3059 1320 TTGCCAGATGATTTCATGGGTTGTGTCCTTGCTTGGAATACTAGGAACATTGATGCTACT
S-040530 1243
I-3059 1380 TCAACTGGTAATTATAATTATAAATATAGGTATCTTAGACATGGCAAGCTTAGGCCCTTT
S-040530 1303
I-3059 1440 GAGAGAGACATATCTAATGTGCCTTTCTCCCCTGATGGCAAACCTTGCACCCCACCTGCT
S-040530 1363
I-3059 1500 CTTAATTGTTATTGGCCATTAAATGATTATGGTTTTTACACCACTACTGGCATTGGCTAC
S-040530 1423
I-3059 1560 CAACCTTACAGAGTTGTAGTACTTTCTTTTGAACTTTTAAATGCACCGGCCACGGTTTGT
S-040530 1483
I-3059 1620 GGACCAAAATTATCCACTGACCTTATTAAGAACCAGTGTGTCAATTTTAATTTTAATGGA
S-040530 1543
I-3059 1680 CTCACTGGTACTGGTGTGTTAACTCCTTCTTCA__AAGAGATTTCAACCATTTCAACAAT
S-040530 1603
I-3059 1738 TTGGCCGTGATGTCTCTGATTTCACTGATTCCGTTCGAGATCCTAAAACATCTGAAATAT
S-040530 1661
I-3059 1798 TAGACATTTCACCTTGCTCTTTTGGGGGTGTAAGTGTAATTACACCTGGAACAAATGCTT
S-040530 1721
I-3059 1858 _CATCTGAAGTTGCTGTTCTATATCAAGATGTTAACTGCACTGATGTTTCTACAGCAATC
S-040530 1781
I-3059 1917 CATGCAGATCAACTCACACCAGCTTGGCGCATATATTCTACTGGAAACAATGTATTCCAG
S-040530 1840
I-3059 1977 ACTCAAGCAGGCTGTCTTATAGGAGCTGAGCATGTCGACACTTCTTATGAGTGCGACATT
S-040530 1900
I-3059 2037 CCTATTGGAGCTGGCATTTGTGCTAGTTACCATACAGTTTCTTTATTACGTAGTACTAGC
S-040530 1960
I-3059 2097 CAAAAATCTATTGTGGCTTATACTATGTCTTTAGGTGCTGATAGTTCAATTGCTTACTCT
S-040530 2020
I-3059 2157 AATAACACCATTGCTATACCTACTAACTTTTCAATTAGCATTACTACAGAAGTAATGCCT
S-040530 2080
I-3059 2217 GTTTCTATGGCTAAAACCTCCGTAGATTGTAATATGTACATCTGCGGAGATTCTACTGAA
S-040530 2140
I-3059 2277 TGTGCTAATTTGCTTCTCCAATATGGTAGCTTTTGCACACAACTAAATCGTGCACTCTCA
S-040530 2200
I-3059 2337 GGTATTGCTGCTGAACAGGATCGCAACACACGTGAAGTGTTCGCTCAAGTCAAACAAATG
S-040530 2260
I-3059 2397 TACAAAACCCCAACTTTGAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCTGAC
S-040530 2320
I-3059 2457 CCTCTAAAGCCAACTAAGAGGTCTTTTATTGAGGACTTGCTCTTTAATAAGGTGACACTC
S-040530 2380
I-3059 2517 GCTGATGCTGGCTTCATGAAGCAATATGGCGAATGCCTAGGTGATATTAATGCTAGAGAT
S-040530 2440
I-3059 2577 CTCATTTGTGCGCAGAAGTTCAATGGGCTTACAGTGTTGCCACCTCTGCTCACTGATGAT
S-040530 2500
I-3059 2637 ATGATTGCTGCCTACACTGCTGCTCTAGTTAGTGGTACTGCCACTGCTGGATGGACATTT
S-040530 2560

FIGURE 32.2

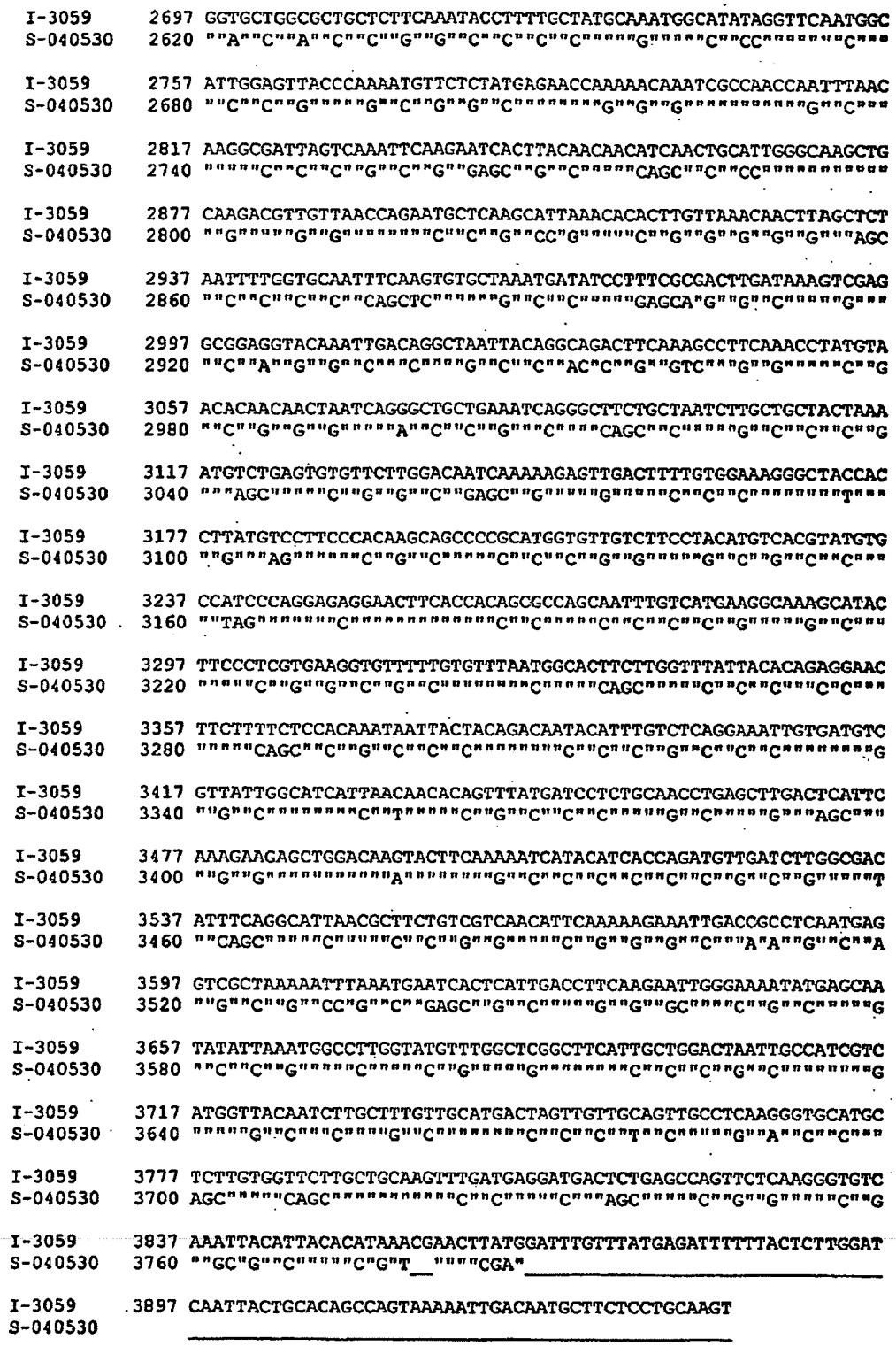

I-3059 2697 GGTGCTGGCGCTGCTCTTCAAATACCTTTTGCTATGCAAATGGCATATAGGTTCAATGGC
S-040530 2620
I-3059 2757 ATTGGAGTTACCCAAAATGTTCTCTATGAGAACCAAAAACAAATCGCCAACCAATTTAAC
S-040530 2680
I-3059 2817 AAGGCGATTAGTCAAATTCAAGAATCACTTACAACAACATCAACTGCATTGGGCAAGCTG
S-040530 2740
I-3059 2877 CAAGACGTTGTTAACCAGAATGCTCAAGCATTAAACACACTTGTTAAACAACTTAGCTCT
S-040530 2800
I-3059 2937 AATTTTGGTGCAATTTCAAGTGTGCTAAATGATATCCTTTCGCGACTTGATAAAGTCGAG
S-040530 2860
I-3059 2997 GCGGAGGTACAAATTGACAGGCTAATTACAGGCAGACTTCAAAGCCTTCAAACCTATGTA
S-040530 2920
I-3059 3057 ACACAACAACTAATCAGGGCTGCTGAAATCAGGGCTTCTGCTAATCTTGCTGCTACTAAA
S-040530 2980
I-3059 3117 ATGTCTGAGTGTGTTCTTGGACAATCAAAAAGAGTTGACTTTTGTGGAAAGGGCTACCAC
S-040530 3040
I-3059 3177 CTTATGTCCTTCCCACAAGCAGCCCCGCATGGTGTTGTCTTCCTACATGTCACGTATGTG
S-040530 3100
I-3059 3237 CCATCCCAGGAGAGGAACTTCACCACAGCGCCAGCAATTTGTCATGAAGGCAAAGCATAC
S-040530 3160
I-3059 3297 TTCCCTCGTGAAGGTGTTTTTGTGTTTAATGGCACTTCTTGGTTTATTACACAGAGGAAC
S-040530 3220
I-3059 3357 TTCTTTTCTCCACAAATAATTACTACAGACAATACATTTGTCTCAGGAAATTGTGATGTC
S-040530 3280
I-3059 3417 GTTATTGGCATCATTAACAACACAGTTTATGATCCTCTGCAACCTGAGCTTGACTCATTC
S-040530 3340
I-3059 3477 AAAGAAGAGCTGGACAAGTACTTCAAAAATCATACATCACCAGATGTTGATCTTGGCGAC
S-040530 3400
I-3059 3537 ATTTCAGGCATTAACGCTTCTGTCGTCAACATTCAAAAAGAAATTGACCGCCTCAATGAG
S-040530 3460
I-3059 3597 GTCGCTAAAAATTTAAATGAATCACTCATTGACCTTCAAGAATTGGGAAAATATGAGCAA
S-040530 3520
I-3059 3657 TATATTAAATGGCCTTGGTATGTTTGGCTCGGCTTCATTGCTGGACTAATTGCCATCGTC
S-040530 3580
I-3059 3717 ATGGTTACAATCTTGCTTTGTTGCATGACTAGTTGTTGCAGTTGCCTCAAGGGTGCATGC
S-040530 3640
I-3059 3777 TCTTGTGGTTCTTGCTGCAAGTTTGATGAGGATGACTCTGAGCCAGTTCTCAAGGGTGTC
S-040530 3700
I-3059 3837 AAATTACATTACACATAAACGAACTTATGGATTTGTTTATGAGATTTTTTACTCTTGGAT
S-040530 3760
I-3059 3897 CAATTACTGCACAGCCAGTAAAAATTGACAATGCTTCTCCTGCAAGT
S-040530

FIGURE 32.3

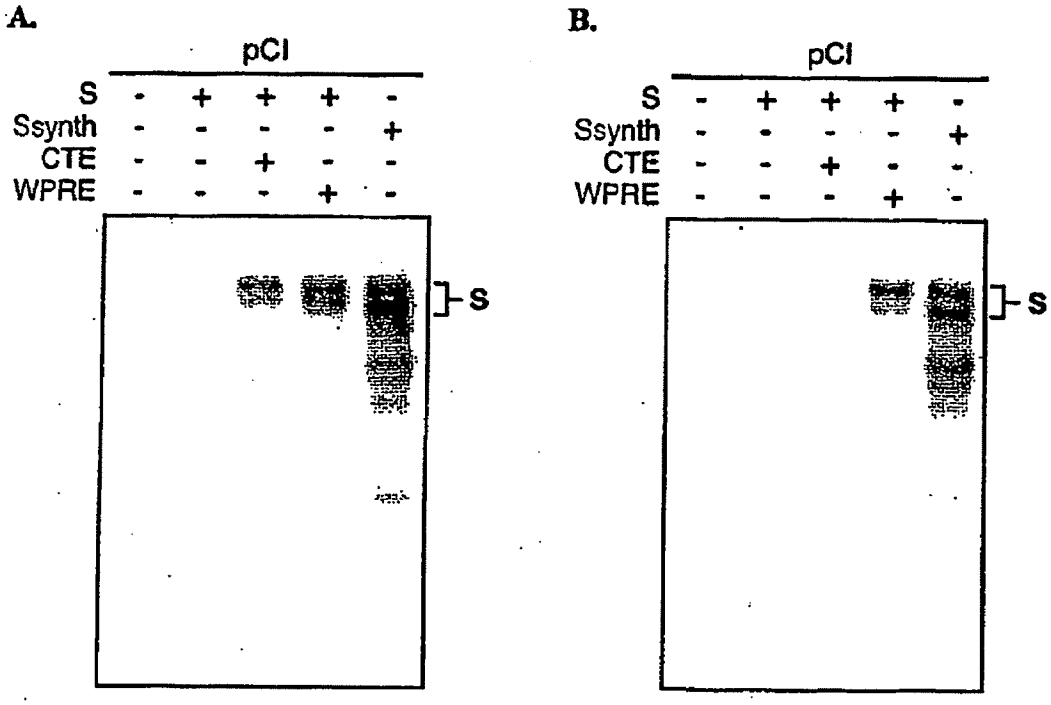
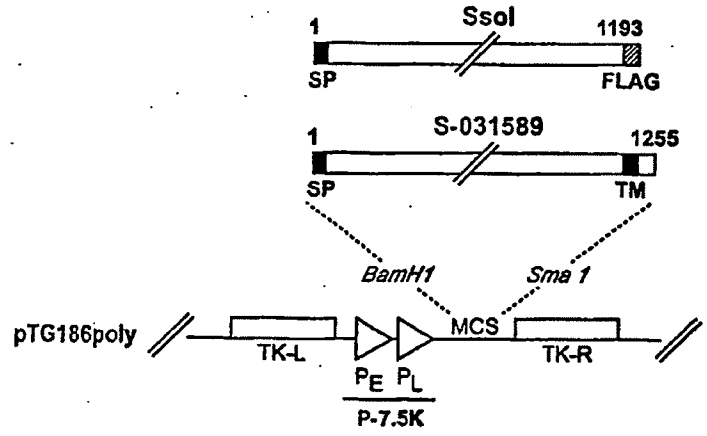
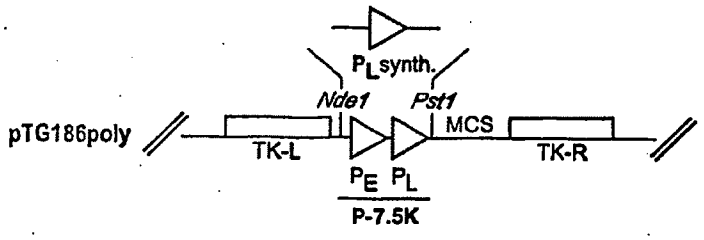


FIGURE 33

A.



B.



C.

CATATG AGC $[T]_{20}$GGCATATAAATA GACTC GGCGCGCC AT CTGCAG
Nde1 promoteur 480 *Asc1* *Pst1*

FIGURE 34 A-C

D.

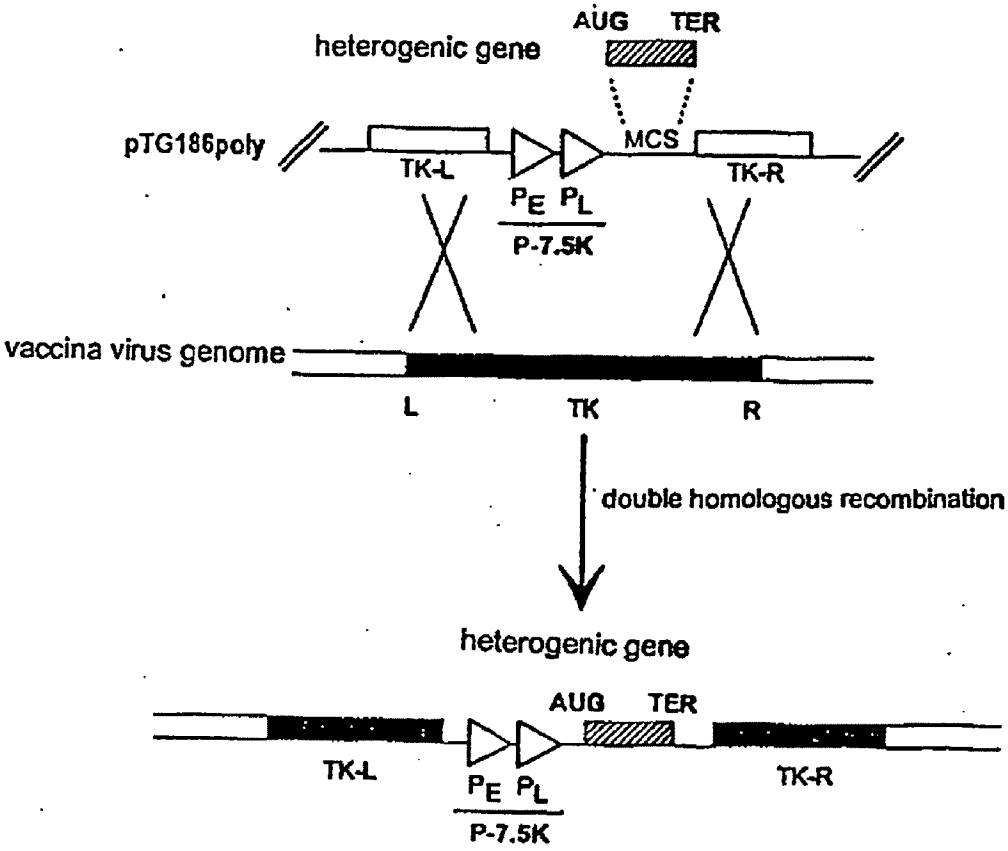

AUG
TER
heterogenic gene
MCS
pTG186poly
TK-L
TK-R
P_E P_L
P-7.5K
vaccina virus genome
L
TK
R
double homologous recombination
heterogenic gene
AUG
TER
TK-L
TK-R
P_E P_L
P-7.5K

FIGURE 34 D

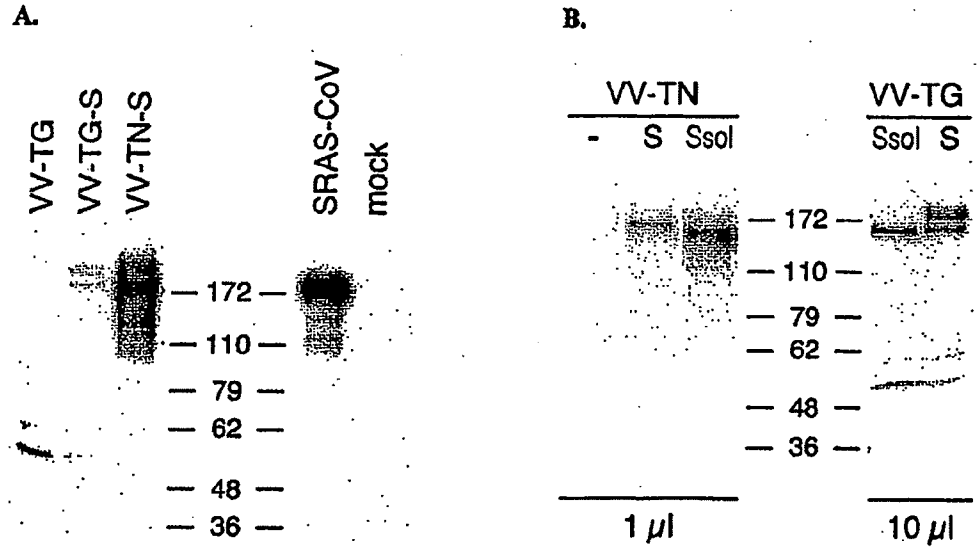
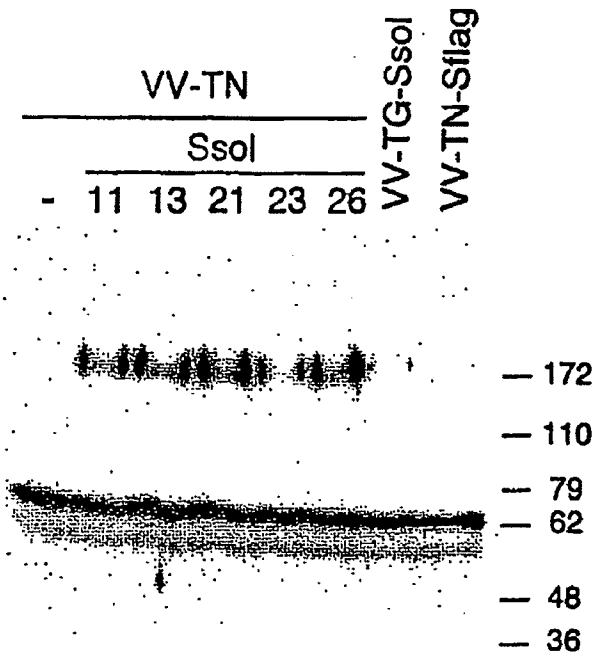

A.
VV-TG
VV-TG-S
VV-TN-S
SRAS-CoV
mock
172
110
79
62
48
36
B.
VV-TN
- S Ssol
VV-TG
Ssol S
172
110
79
62
48
36
1 µl
10 µl

FIGURE 35

A.


VV-TN
SsoI
- 11 13 21 23 26
VV-TG-SsoI
VV-TN-Sflag
172
110
79
62
48
36

B.

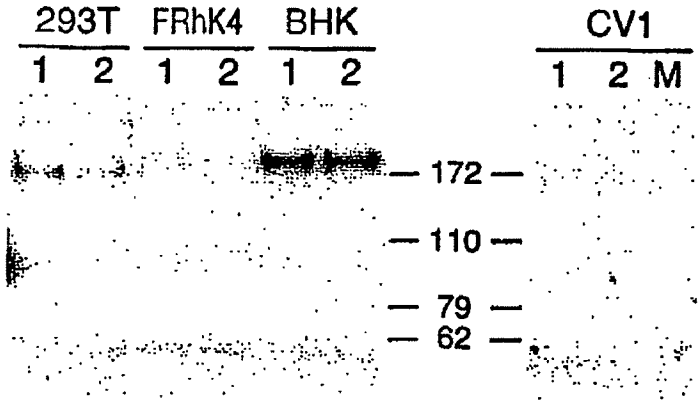

293T
FRhK4
BHK
CV1
1 2 1 2 1 2 1 2 M
172
110
79
62

FIGURE 36

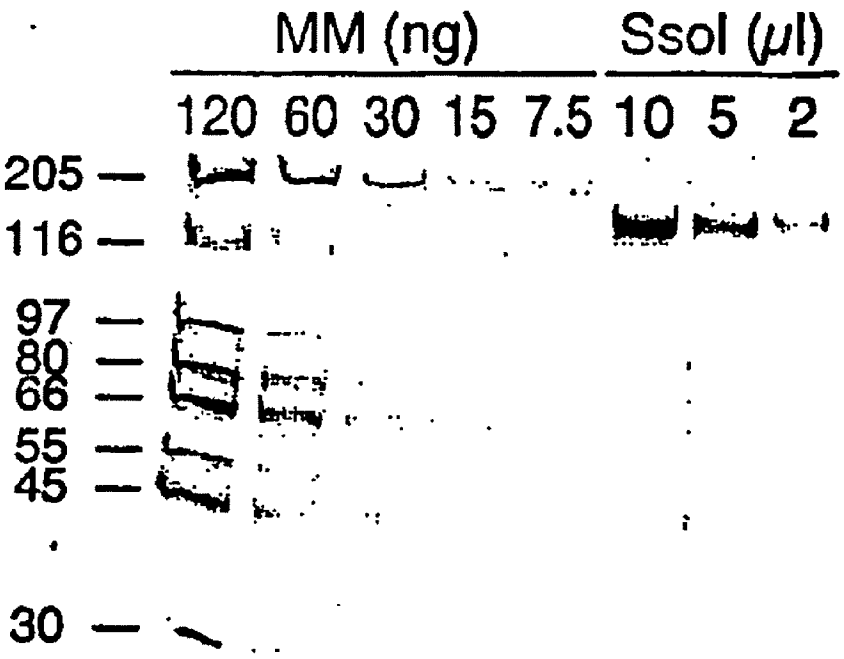


FIGURE 37

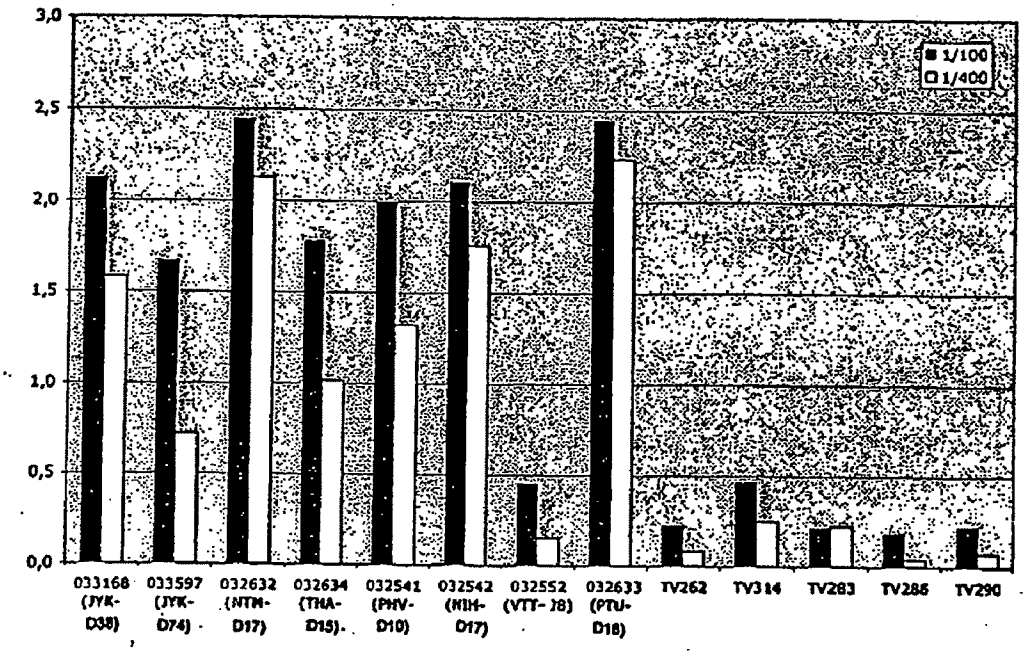
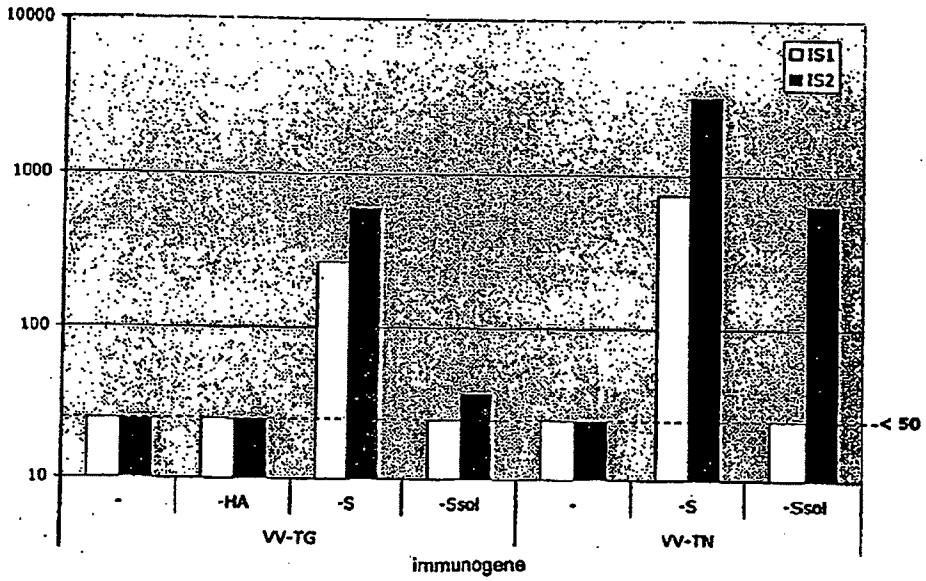

3,0
2,5
2,0
1,5
1,0
0,5
0,0
1/100
1/400
033168 (JYK-D38)
033597 (JYK-D74)
032632 (NTH-D17)
032634 (THA-D15)
032541 (PHV-D10)
032542 (NIH-D17)
032552 (VTT-38)
032633 (PTU-D18)
TV262
TV314
TV283
TV286
TV290
serums

FIGURE 38

A.


10000
1000
100
10
IS1
IS2
< 50
-
-HA
-S
-Ssol
-
-S
-Ssol
VV-TG
VV-TN
Immunogene

B.

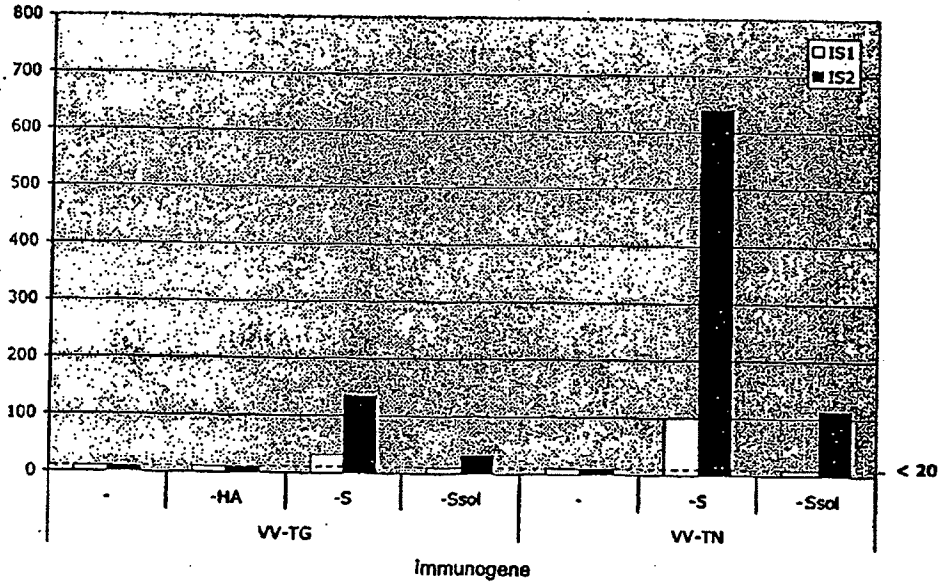

800
700
600
500
400
300
200
100
0
IS1
IS2
< 20
-
-HA
-S
-Ssol
-
-S
-Ssol
VV-TG
VV-TN
Immunogene

FIGURE 39

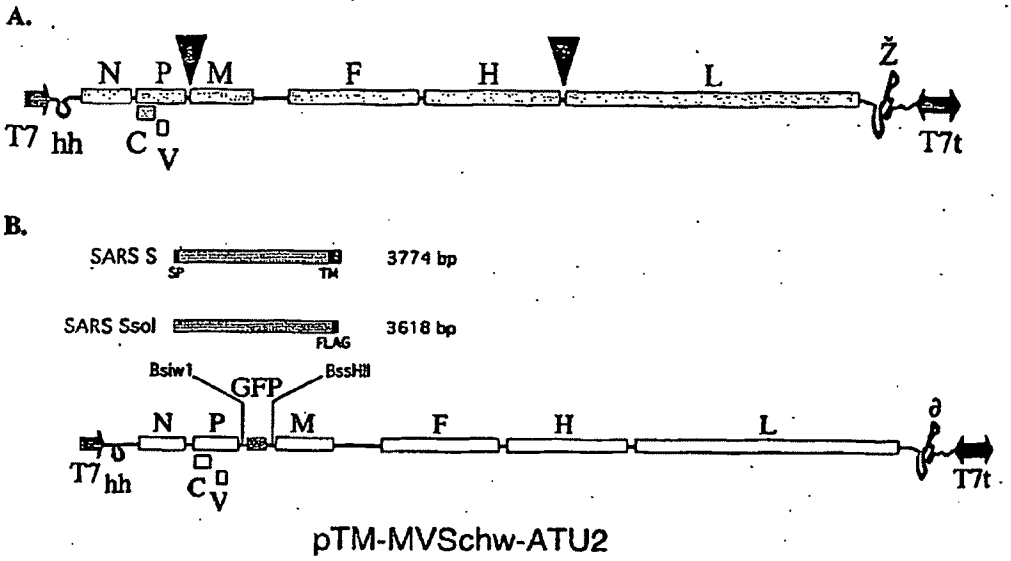


FIGURE 40

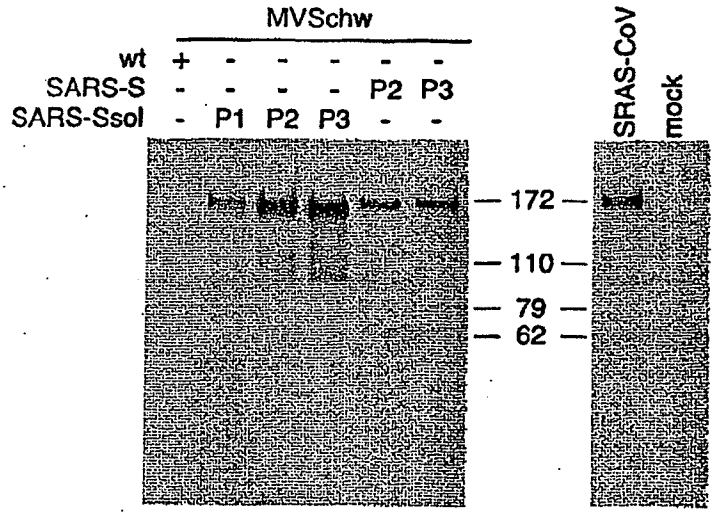


FIGURE 41

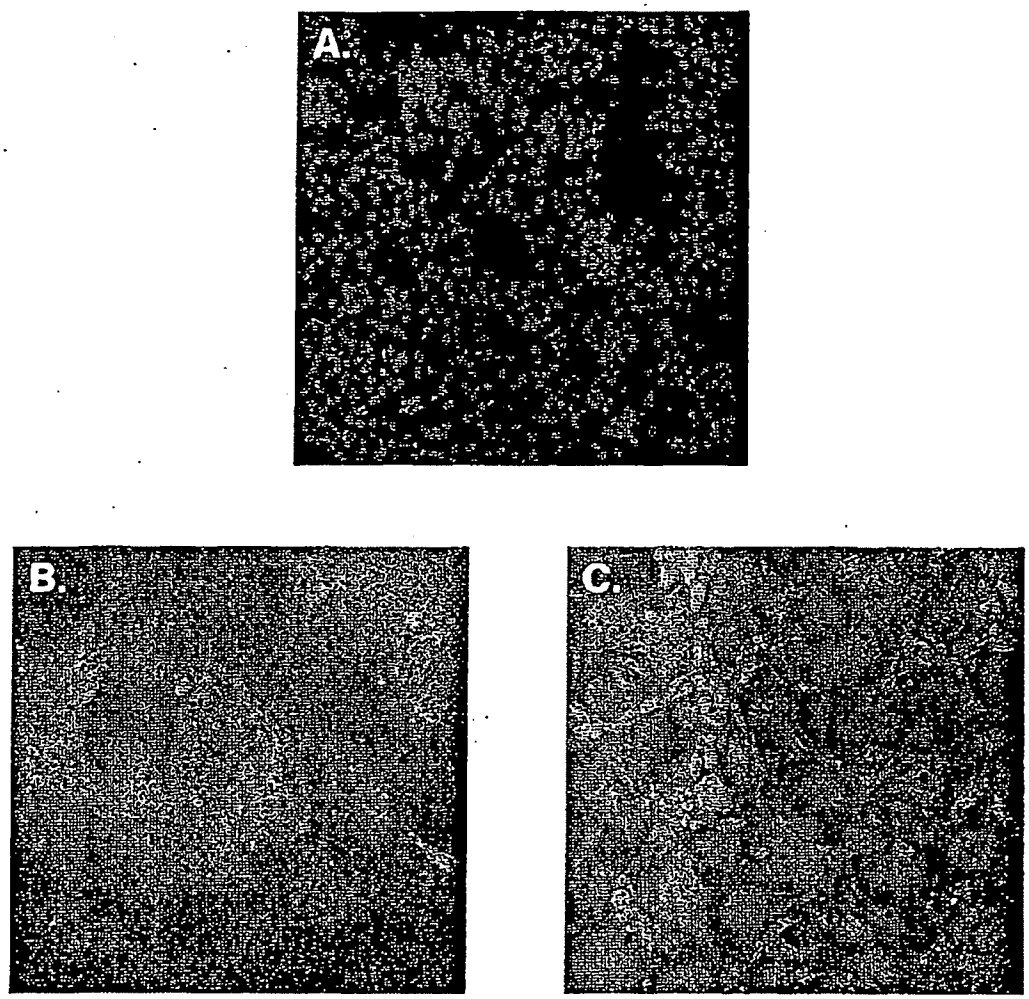


FIGURE 42

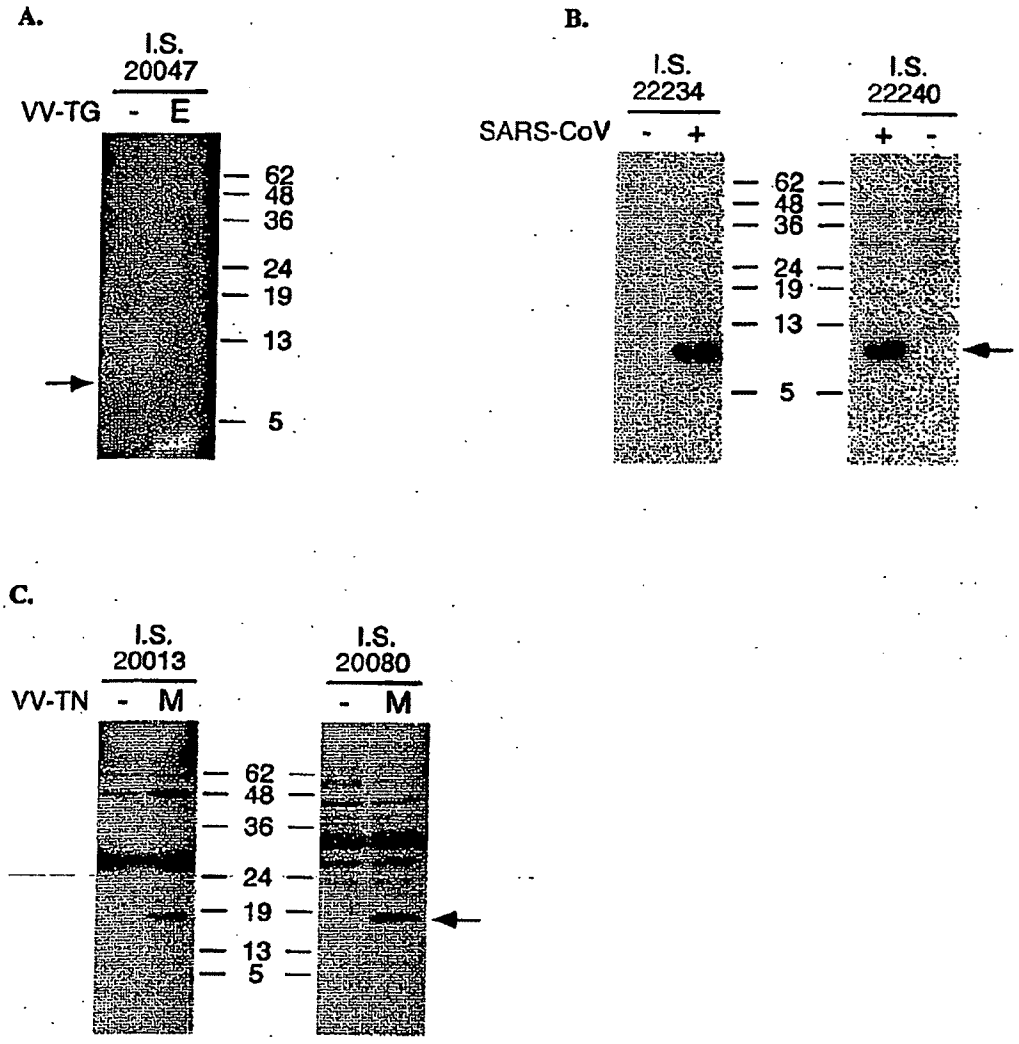

A.
I.S. 20047
VV-TG - E
62
48
36
24
19
13
5
B.
I.S. 22234
I.S. 22240
SARS-CoV - + + -
62
48
36
24
19
13
5
C.
I.S. 20013
I.S. 20080
VV-TN - M - M
62
48
36
24
19
13
5

FIGURE 43

USE OF PROTEINS AND PEPTIDES ENCODED BY THE GENOME OF A NOVEL SARS-ASSOCIATED CORONAVIRUS STRAIN

[0001] The present invention relates to a novel strain of severe acute respiratory syndrome (SARS)-associated coronavirus derived from a sample recorded under No. 031589 and collected in Hanoi (Vietnam), to nucleic acid molecules derived from its genome, to the proteins and peptides encoded by said nucleic acid molecules and to their applications, in particular as diagnostic reagents and/or as vaccine.

[0002] Coronavirus is a virus containing single-stranded RNA, of positive polarity, of approximately 30 kilobases which replicates in the cytoplasm of the host cells; the 5' end of the genome has a capped structure and the 3' end contains a polyA tail. This virus is enveloped and comprises, at its surface, peplomeric structures called spicules.

[0003] The genome comprises the following open reading frames or ORFs, from its 5' end to its 3' end: ORF1a and ORF1b corresponding to the proteins of the transcription-replication complex, and ORF-S, ORF-E, ORF-M and ORF-N corresponding to the structural proteins S, E, M and N. It also comprises ORFs corresponding to proteins of unknown function encoded by: the region situated between ORF-S and ORF-E and overlapping the latter, the region situated between ORF-M and ORF-N, and the region included in ORF-N.

[0004] The S protein is a membrane glycoprotein (200-220 kDa) which exists in the form of spicules or spikes emerging from the surface of the viral envelope. It is responsible for the attachment of the virus to the receptors of the host cell and for inducing the fusion of the viral envelope with the cell membrane.

[0005] The small envelope protein (E), also called sM (small membrane), which is a nonglycosylated transmembrane protein of about 10 kDa, is the protein present in the smallest quantity in the virion. It plays a powerful role in the coronavirus budding process which occurs at the level of the intermediate compartment in the endoplasmic reticulum and the Golgi apparatus.

[0006] The M protein or matrix protein (25-30 kDa) is a more abundant membrane glycoprotein which is integrated into the viral particle by an M/E interaction, whereas the incorporation of S into the particles is directed by an S/M interaction. It appears to be important for the viral maturation of coronaviruses and for the determination of the site where the viral particles are assembled.

[0007] The N protein or nucleocapsid protein (45-50 kDa) which is the most conserved among the coronavirus structural proteins is necessary for encapsidating the genomic RNA and then for directing its incorporation into the virion. This protein is probably also involved in the replication of the RNA.

[0008] When the host cell is infected, the reading frame (ORF) situated in 5' of the viral genome is translated into a polyprotein which is cleaved by the viral proteases and then releases several nonstructural proteins such as the RNA-dependent RNA polymerase (Rep) and the ATPase helicase (Hel). These two proteins are involved in the replication of the viral genome and in the generation of transcripts which are used in the synthesis of the viral proteins. The mechanisms by which these subgenomic mRNAs are produced are not completely understood; however, recent facts indicate that the sequences for regulation of transcription at the 5' end of each gene represent signals which regulate the discontinuous transcription of the subgenomic mRNAs.

[0009] The proteins of the viral membrane (S, E and M proteins) are inserted into the intermediate compartment, whereas the replicated RNA (+ strand) is assembled with the N (nucleocapsid) protein. This protein-RNA complex then combines with the M protein contained in the membranes of the endoplasmic reticulum and the viral particles form when the nucleocapsid complex buds into the endoplasmic reticulum. The virus then migrates across the Golgi complex and eventually leaves the cell, for example by exocytosis. The site of attachment of the virus to the host cell is at the level of the S protein.

[0010] Coronaviruses are responsible for 15 to 30% of colds in humans and for respiratory and digestive infections in animals, especially cats (FIPV: Feline infectious peritonitis virus), poultry (IBV: Avian infectious bronchitis virus), mice (MHV: Mouse hepatitis virus), pigs (TGEV: Transmissible gastroenteritis virus, PEDV: Porcine Epidemic diarrhea virus, PRCoV: Porcine Respiratory Coronavirus, HEV: Hemagglutinating encephalomyelitis Virus) and bovines (BCoV: Bovine coronavirus).

[0011] In general, each coronavirus affects only one species; in immunocompetent individuals, the infection induces optionally neutralizing antibodies and cell immunity, capable of destroying the infected cells.

[0012] An epidemy of atypical pneumonia, called severe acute respiratory syndrome (SARS) has spread in various countries (Vietnam, Hong Kong, Singapore, Thailand and Canada) during the first quarter of 2003, from an initial focus which appeared in China in the last quarter of 2002. The severity of this disease is such that its mortality rate is about 3 to 6%. The determination of the causative agent of this disease is underway by numerous laboratories worldwide.

[0013] In March 2003, a new coronavirus (SARS-CoV or SARS virus) was isolated, in association with cases of severe acute respiratory syndrome (T. G. KSIAZEK et al., The New England Journal of Medicine, 2003, 348, 1319-1330; C. DROSTEN et al., The New England Journal of Medicine, 2003, 348, 1967-1976; Peiris et al., Lancet, 2003, 361, 1319).

[0014] Genomic sequences of this new coronavirus have thus been obtained, in particular those of the Urbani isolate (Genbank accession No. AY274119.3 and A. MARRA et al., Science, May 1, 2003, 300, 1399-1404) and the Toronto isolate (Tor2, Genbank accession No. AY278741 and A. ROTA et al., Science, 2003, 300, 1394-1399).

[0015] The organization of the genome is comparable with that of other known coronaviruses, thus making it possible to confirm that SARS-CoV belongs to the Coronaviridae family; open reading frames ORF1a and 1b and open reading frames corresponding to the S, E, M and N proteins, and to proteins encoded by: the region situated between ORF-S and ORF-E (ORF3), the region situated between ORF-S and ORF-E and overlapping ORF-E (ORF4), the region situated between ORF-M and ORF-N (ORF7 to

ORF11) and the region corresponding to ORF-N (ORF13 and ORF14), have in particular been identified.

[0016] Seven differences have been identified between the sequences of the Tor2 and Urbani isolates; 3 correspond to silent mutations (c/t at position 16622 and a/g at position 19064 of ORF1b, t/c at position 24872 of ORF-S) and 4 modify the amino acid sequence of respectively: the proteins encoded by ORF1a (c/t at position 7919 corresponding to the A/V mutation), the S protein (g/t at position 23220 corresponding to the A/S mutation), the protein encoded by ORF3 (a/g at position 25298 corresponding to the R/G mutation) and the M protein (t/c at position 26857 corresponding to the S/P mutation).

[0017] In addition, phylogenetic analysis shows that SARS-CoV is distant from other coronaviruses and that it did not appear by mutation of human respiratory coronaviruses nor by recombination between known coronaviruses (for a review, see Holmes, J. C. I., 2003, 111, 1605-1609).

[0018] The determination and the taking into account of new variants are important for the development of reagents for the detection and diagnosis of SARS which are sufficiently sensitive and specific, and immunogenic compositions capable of protecting populations against epidemics of SARS.

[0019] The inventors have now identified another strain of SARS-associated coronavirus which is distinguishable from the Tor2 and Urbani isolates.

[0020] The subject of the present invention is therefore an isolated or purified strain of severe acute respiratory syndrome-associated human coronavirus, characterized in that its genome has, in the form of complementary DNA, a serine codon at position 23220-23222 of the gene for the S protein or a glycine codon at position 25298-25300 of the gene for ORF3, and an alanine codon at position 7918-7920 of ORF1a or a serine codon at position 26857-26859 of the gene for the M protein, said positions being indicated in terms of reference to the Genbank sequence AY274119.3.

[0021] According to an advantageous embodiment of said strain, the DNA equivalent of its genome has a sequence corresponding to the sequence SEQ ID No: 1; this coronavirus strain is derived from the sample collected from the bronchoaleveolar washings from a patient suffering from SARS, recorded under the No. 031589 and collected at the Hanoi (Vietnam) French hospital.

[0022] In accordance with the invention, said sequence SEQ ID No: 1 is that of the deoxyribonucleic acid corresponding to the ribonucleic acid molecule of the genome of the isolated coronavirus strain as defined above.

[0023] The sequence SEQ ID No: 1 is distinguishable from the Genbank sequence AY274119.3 (Tor2 isolate) in that it possesses the following mutations:

[0024] g/t at position 23220; the alanine codon (gct) at position 577 of the amino acid sequence of the Tor2 S protein is replaced by a serine codon (tct),

[0025] a/g at position 25298; the arginine codon (aga) at position 11 of the amino acid sequence of the protein encoded by the Tor2 ORF3 is replaced by a glycine codon (gga).

[0026] In addition, the sequence SEQ ID No: 1 is distinguishable from the Genbank sequence AY278741 (Urbani isolate) in that it possesses the following mutations:

[0027] t/c at position 7919; the valine codon (gtt) in position 2552 of the amino acid sequence of the protein encoded by ORF1a is replaced by an alanine codon (gct),

[0028] t/c at position 16622: this mutation does not modify the amino acid sequence of the proteins encoded by ORF1b (silent mutation),

[0029] g/a at position 19064: this mutation does not modify the amino acid sequence of the proteins encoded by ORF1b (silent mutation),

[0030] c/t at position 24872: this mutation does not modify the amino acid sequence of the S protein, and c/t at position 26857: the proline codon (ccc) at position 154 of the amino acid sequence of the M protein is replaced by a serine codon (tcc).

[0031] Unless otherwise stated, the positions of the nucleotide and peptide sequences are indicated with reference to the Genbank sequence AY274119.3.

[0032] The subject of the present invention is also an isolated or purified polynucleotide, characterized in that its sequence is that of the genome of the isolated coronavirus strain as defined above.

[0033] According to an advantageous embodiment of said polynucleotide, it has the sequence SEQ ID No: 1.

[0034] The subject of the present invention is also an isolated or purified polynucleotide, characterized in that its sequence hybridizes under high stringency conditions with the sequence of the polynucleotide as defined above.

[0035] The terms "isolated or purified" mean modified "by the hand of humans" from the natural state; in other words if an object exists in nature, it is said to be isolated or purified if it is modified or extracted from its natural environment or both. For example, a polynucleotide or a protein/peptide naturally present in a living organism is neither isolated nor purified; on the other hand, the same polynucleotide or protein/peptide separated from coexisting molecules in its natural environment, obtained by cloning, amplification and/or chemical synthesis is isolated for the purposes of the present invention. Furthermore, a polynucleotide or a protein/peptide which is introduced into an organism by transformation, genetic manipulation or by any other method, is "isolated" even if it is present in said organism. The term purified as used in the present invention means that the proteins/peptides according to the invention are essentially free of association with the other proteins or polypeptides, as is for example the product purified from the culture of recombinant host cells or the product purified from a nonrecombinant source.

[0036] For the purposes of the present invention, high stringency hybridization conditions are understood to mean temperature and ionic strength conditions chosen such that they make it possible to maintain the specific and selective hybridization between complementary polynucleotides.

[0037] By way of illustration, high stringency conditions for the purposes of defining the above polynucleotides are advantageously the following: the DNA-DNA or DNA-

RNA hybridization is performed in two steps: (1) prehybridization at 42° C. for 3 hours in phosphate buffer (20 mM, pH 7.5) containing 5×SSC (1×SSC corresponds to a 0.15 M NaCl+0.015 M sodium citrate solution), 50% formamide, 7% sodium dodecyl sulfate (SDS), 10×Denhardt's, 5% dextran sulfate and 1% salmon sperm DNA; (2) hybridization for 20 hours at 42° C. followed by 2 washings of 20 minutes at 20° C. in 2×SSC+2% SDS, 1 washing of 20 minutes at 20° C. in 0.1×SSC+0.1% SDS. The final washing is performed in 0.1×SSC+0.1% SDS for 30 minutes at 60° C.

[0038] The subject of the present invention is also a representative fragment of the polynucleotide as defined above, characterized in that it is capable of being obtained either by the use of restriction enzymes whose recognition and cleavage sites are present in said polynucleotide as defined above, or by amplification with the aid of oligonucleotide primers specific for said polynucleotide as defined above, or by transcription in vitro, or by chemical synthesis.

[0039] According to an advantageous embodiment of said fragment, it is selected from the group consisting of: the cDNA corresponding to at least one open reading frame (ORF) chosen from: ORF1a, ORF1b, ORF-S, ORF-E, ORF-M, ORF-N, ORF3, ORF4, ORF7 to ORF11, ORF13 and ORF14 and the cDNA corresponding to the noncoding 5' or 3' ends of said polynucleotide.

[0040] According to an advantageous feature of this embodiment, said fragment has a sequence selected from the group consisting of:

[0041] the sequences SEQ ID NO: 2 and 4 representing the cDNA corresponding to the ORF-S which encodes the S protein,

[0042] the sequences SEQ ID NO: 13 and 15 representing the cDNA corresponding to the ORF-E which encodes the E protein,

[0043] the sequences SEQ ID NO: 1-6 and 18 representing the cDNA corresponding to the ORF-M which encodes the M protein,

[0044] the sequences SEQ ID NO: 36 and 38 representing the cDNA corresponding to the ORF-N which encodes the N protein,

[0045] the sequences representing the cDNA corresponding respectively: to ORF1a and ORF1b (ORF1ab, SEQ ID NO: 31), to ORF3 and ORF4 (SEQ ID NO: 7, 8), to ORF7 to 11 (SEQ ID NO: 19, 20) to ORF13 (SEQ ID NO: 32) and to ORF14 (SEQ ID NO: 34), and

[0046] the sequences representing the cDNAs corresponding respectively to the noncoding 5' (SEQ ID NO: 39 and 72) and 3' (SEQ ID NO: 40, 73) ends of said polynucleotide.

[0047] The subject of the present invention is also a cDNA fragment encoding the S protein, as defined above, characterized in that it has a sequence selected from the group consisting of the sequences SEQ ID NO: 5 and 6 (Sa and Sb fragments).

[0048] The subject of the present invention is also a cDNA fragment corresponding to ORF1a and ORF1b as defined above, characterized in that it has a sequence selected from the group consisting of the sequences SEQ ID NO: 41 to 54 (L0 to L12 fragments).

[0049] The subject of the present invention is also a polynucleotide fragment as defined above, characterized in that it has at least 15 consecutive bases or base pairs of the sequence of the genome of said strain including at least one of those situated in position 7979, 16622, 19064, 23220, 24872, 25298 and 26857. Preferably this is a fragment of 20 to 2500 bases or base pairs, preferably from 20 to 400.

[0050] According to an advantageous embodiment of said fragment, it includes at least one pair of bases or base pairs corresponding to the following positions: 7919 and 23220, 7919 and 25298, 16622 and 23220, 19064 and 23220, 16622 and 25298, 19064 and 25298, 23220 and 24872, 23220 and 26857, 24872 and 25298, 25298 and 26857.

[0051] The subject of the present invention is also primers of at least 18 bases capable of amplifying a fragment of the genome of a SARS-associated coronavirus or of the DNA equivalent thereof.

[0052] According to an embodiment of said primers, they are selected from the group consisting of:

[0053] the pair of primers No. 1 corresponding respectively to positions 28507 to 28522 (sense primer, SEQ ID NO: 60) and 28774 to 28759 (antisense primer, SEQ ID NO: 61) of the sequence of the polynucleotide as defined above,

[0054] the pair of primers No. 2 corresponding respectively to positions 28375 to 28390 (sense primer, SEQ ID NO: 62) and 28702 to 28687 (antisense primer, SEQ ID NO: 63) of the sequence of the polynucleotide as defined above, and

[0055] the pair of primers consisting of the primers SEQ ID Nos: 55 and 56.

[0056] The subject of the present invention is also a probe capable of detecting the presence of the genome of a SARS-associated coronavirus or of a fragment thereof, characterized in that it is selected from the group consisting of: the fragments as defined above and the fragments corresponding to the following positions of the polynucleotide sequence as defined above: 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 (SEQ ID NO: 64 to 67).

[0057] The probes and primers according to the invention may be labeled directly or indirectly with a radioactive or nonradioactive compound by methods well known to persons skilled in the art so as to obtain a detectable and/or quantifiable signal. Among the radioactive isotopes used, there may be mentioned ^{32}P, ^{33}P, ^{35}S, ^{3}H or ^{125}I. The nonradioactive entities are selected from ligands such as biotin, avidin, streptavidin, digoxygenin, haptens, dyes, luminescent agents such as radioluminescent, chemoluminescent, bioluminescent, fluorescent and phosphorescent agents.

[0058] The invention encompasses the labeled probes and primers derived from the preceding sequences.

[0059] Such probes and primers are useful for the diagnosis of infection by a SARS-associated coronavirus.

[0060] The subject of the present invention is also a method for the detection of a SARS-associated coronavirus, from a biological sample, which method is characterized in that it comprises at least:

[0061] (a) the extraction of nucleic acids present in said biological sample,

[0062] (b) the amplification of a fragment of ORF-N by RT-PCR with the aid of a pair of primers as defined above, and

[0063] (c) the detection, by any appropriate means, of the amplification products obtained in (b).

[0064] The amplification products (amplicons) in (b) are 268 bp for the pair of primers No. 1 and 328 bp for the pair of primers No. 2.

[0065] According to an advantageous embodiment of said method, the step (b) of detection is carried out with the aid of at least one probe corresponding to positions 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 of the sequence of the polynucleotide as defined above.

[0066] Preferably, the SARS-associated coronavirus genome is detected and optionally quantified by PCR in real time with the aid of the pair of primers No. 2 and probes corresponding to positions 28541 to 28563 and 28565 to 28589 labeled with different compounds, in particular different fluorescent agents.

[0067] The real time RT-PCR which uses this pair of primers and this probe is very sensitive since it makes it possible to detect 102 copies of RNA and up to 10 copies of RNA; it is in addition reliable and reproducible.

[0068] The invention encompasses the single-stranded, double-stranded and triple-stranded polydeoxyribonucleotides and polyribonucleotides corresponding to the sequence of the genome of the isolated strain of coronavirus and its fragments as defined above, and to their sense or antisense complementary sequences, in particular the RNAs and cDNAs corresponding to the sequence of the genome and of its fragments as defined above.

[0069] The present invention also encompasses the amplification fragments obtained with the aid of primers specific for the genome of the purified or isolated strain as defined above, in particular with the aid of primers or pairs of primers as defined above, the restriction fragments formed by or comprising the sequence of fragments as defined above, the fragments obtained by transcription in vitro from a vector containing the sequence SEQ ID NO: 1 or a fragment as defined above, and fragments obtained by chemical synthesis. Examples of restriction fragments are deduced from the restriction map of the sequence SEQ ID NO: 1 illustrated by FIG. **13**. In accordance with the invention, said fragments are either in the form of isolated fragments, or in the form of mixtures of fragments. The invention also encompasses fragments modified, in relation to the preceding ones, by removal or addition of nucleotides in a proportion of about 15%, relative to the length of the above fragments and/or modified in terms of the nature of the nucleotides, as long as the modified nucleotide fragments retain a capacity for hybridization with the genomic or antigenomic RNA sequences of the isolate as defined above.

[0070] The nucleic acid molecules according to the invention are obtained by conventional methods, known per se, following standard protocols such as those described in *Current Protocols in Molecular Biology* (Frederick M. AUSUBEL, 2000, Wiley and son Inc., Library of Congress, USA). For example, they may be obtained by amplification of a nucleic sequence by PCR or RT-PCR or alternatively by total or partial chemical synthesis.

[0071] The subject of the present invention is also a DNA or RNA chip or filter, characterized in that it comprises at least one polynucleotide or one of its fragments as defined above.

[0072] The DNA or RNA chips or filters according to the invention are prepared by conventional methods, known per se, such as for example chemical or electrochemical grafting of oligonucleotides on a glass or nylon support.

[0073] The subject of the present invention is also a recombinant cloning and/or expression vector, in particular a plasmid, a virus, a viral vector or a phage comprising a nucleic acid fragment as defined above. Preferably, said recombinant vector is an expression vector in which said nucleic acid fragment is placed under the control of appropriate elements for regulating transcription and translation. In addition, said vector may comprise sequences (tags) fused in phase with the 5' and/or 3' end of said insert, which are useful for the immobilization and/or detection and/or purification of the protein expressed from said vector.

[0074] These vectors are constructed and introduced into host cells by conventional recombinant DNA and genetic engineering methods which are known per se. Numerous vectors into which a nucleic acid molecule of interest may be inserted in order to introduce it and to maintain it in a host cell are known per se; the choice of an appropriate vector depends on the use envisaged for this vector (for example replication of the sequence of interest, expression of this sequence, maintenance of the sequence in extrachromosomal form or alternatively integration into the chromosomal material of the host), and on the nature of the host cell.

[0075] In accordance with the invention, said plasmid is selected in particular from the following plasmids:

[0076] the plasmid, called SARS-S, contained in the bacterial strain deposited under the No. I-3059, on Jun. 20, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, said sequence corresponding to the nucleotides at positions 21406 to 25348 (SEQ ID NO: 4), with reference to the Genbank sequence AY274119.3,

[0077] the plasmid, called SARS-S1, contained in the bacterial strain deposited under the No. I-3020, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 5' fragment of the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment corresponding to the nucleotides at positions 21406 to 23454 (SEQ ID NO: 5), with reference to the Genbank sequence AY274119.3 Tor2,

[0078] the plasmid, called SARS-S2, contained in the bacterial strain deposited under the No. I-3019, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 3' fragment of the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the number No. 031589, as defined above, said fragment corresponding to the nucleotides at positions 23322 to 25348 (SEQ ID NO: 6), with reference to the Genbank sequence accession No. AY274119.3,

[0079] the plasmid, called SARS-SE, contained in the bacterial strain deposited under the No. I-3126, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the region situated between ORF-S and ORF-E and overlapping ORF-E of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said region corresponding to the nucleotides at positions 25110 to 26244 (SEQ ID NO: 8), with reference to the Genbank sequence accession No. AY274119.3,

[0080] the plasmid, called SARS-E, contained in the bacterial strain deposited under the No. I-3046, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the E protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 26082 to 26413 (SEQ ID NO: 15), with reference to the Genbank sequence accession No. AY274119.3,

[0081] the plasmid, called SARS-M, contained in the bacterial strain deposited under the No. I-3047, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above; said sequence corresponding to the nucleotides at positions 26330 to 27098 (SEQ ID NO: 18), with reference to the Genbank sequence accession No. AY274119.3,

[0082] the plasmid, called SARS-MN, contained in the bacterial sequence deposited under the No. I-3125, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the region situated between ORF-M and ORF-N of the SARS-CoV strain derived from the sample recorded under the No. 031589 and collected in Hanoi, as defined above, said sequence corresponding to the nucleotides at positions 26977 to 28218 (SEQ ID NO: 20), with reference to the Genbank accession No. AY274119.3,

[0083] the plasmid, called SARS-N, contained in the bacterial strain deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA encoding the N protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 28054 to 29430 (SEQ ID NO: 38), with reference to the Genbank sequence accession No. AY274119.3; thus, this plasmid comprises an insert of sequence SEQ ID NO: 38 and is contained in a bacterial strain which was deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15,

[0084] the plasmid, called SARS-5'NC, contained in the bacterial strain deposited under the No. I-3124, on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the noncoding 5' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 1 to 204 (SEQ ID NO: 39), with reference to the Genbank sequence accession No. AY274119.3,

[0085] the plasmid called SARS-3'NC, contained in the bacterial strain deposited under the No. I-3123 on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the noncoding 3' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to that situated between the nucleotide and position 28933 to 29727 (SEQ ID NO: 40), with reference to the Genbank sequence accession No. AY274119.3, ends with a series of nucleotides a.,

[0086] the expression plasmid, called pIV2.3N, containing a cDNA fragment encoding a C-terminal fusion of the N protein (SEQ ID NO: 37) with a polyhistidine tag,

[0087] the expression plasmid, called pIV2.3S_C, containing a cDNA fragment encoding a C-terminal fusion of the fragment corresponding to positions 475 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag,

[0088] the expression plasmid, pIV2.3S_L, containing a cDNA fragment encoding a C-terminal fusion of the fragment corresponding to positions 14 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag,

[0089] the expression plasmid, called pIV2.4N, containing a cDNA fragment encoding a N-terminal fusion of the N protein (SEQ ID NO: 3) with a polyhistidine tag,

[0090] the expression plasmid, called pIV2.4S_C or pIV2.4S_1, containing an insert encoding a N-terminal fusion of the fragment corresponding to positions 475 to 1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag, and

[0091] the expression plasmid, called pIV2.4S_L, containing a cDNA fragment encoding an N-terminal fusion of the fragment corresponding to positions 14 to

1193 of the amino acid sequence of the S protein (SEQ ID NO: 3) with a polyhistidine tag.

[0092] According to an advantageous feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited under the No. I-3117, on Oct. 23, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

[0093] According to another advantageous feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited under the No. I-3118, on Oct. 23, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

[0094] According to another feature of the expression plasmid as defined above, it is contained in a bacterial strain which was deposited at the CNCM, 25 rue du Docteur Roux, 75724 Paris Cedex 15 under the following numbers:

[0095] a) strain No. I-3118, deposited on Oct. 23, 2003,

[0096] b) strain No. I-3019, deposited on May 12, 2003,

[0097] c) strain No. I-3020, deposited on May 12, 2003,

[0098] d) strain No. I-3059, deposited on Jun. 20, 2003,

[0099] e) strain No. I-3323, deposited on Nov. 22, 2004,

[0100] f) strain No. I-3324, deposited on Nov. 22, 2004,

[0101] g) strain No. I-3326, deposited on Dec. 1, 2004,

[0102] h) strain No. I-3327, deposited on Dec. 1, 2004,

[0103] i) strain No. I-3332, deposited on Dec. 1, 2004,

[0104] j) strain No. I-3333, deposited on Dec. 1, 2004,

[0105] k) strain No. I-3334, deposited on Dec. 1, 2004,

[0106] l) strain No. I-3335, deposited on Dec. 1, 2004,

[0107] m) strain No. I-3336, deposited on Dec. 1, 2004,

[0108] n) strain No. I-3337, deposited on Dec. 1, 2004,

[0109] o) strain No. I-3338, deposited on Dec. 2, 2004,

[0110] p) strain No. I-3339, deposited on Dec. 2, 2004,

[0111] q) strain No. I-3340, deposited on Dec. 2, 2004,

[0112] r) strain No. I-3341, deposited on Dec. 2, 2004.

[0113] The subject of the present invention is also a nucleic acid insert of viral origin, characterized in that it is contained in any of the strains as defined above in a)-r).

[0114] The subject of the present invention is also a nucleic acid containing a synthetic gene allowing optimized expression of the S protein in eukaryotic cells, characterized in that it possesses the sequence SEQ ID NO: 140.

[0115] The subject of the present invention is also an expression vector containing a nucleic acid containing a synthetic gene allowing optimized expression of the S protein, which vector is contained in the bacterial strain deposited at the CNCM, on Dec. 1, 2004, under the No. I-3333.

[0116] According to one embodiment of said expression vector, it is a viral vector, in the form of a viral particle or in the form of a recombinant genome.

[0117] According to an advantageous feature of this embodiment, this is a recombinant viral particle or a recombinant viral genome capable of being obtained by transfection of a plasmid according to paragraphs g), h) and k) to r) as defined above, in an appropriate cellular system, that is to say, for example, cells transfected with one or more other plasmids intended to transcomplement certain functions of the virus that are deleted in the vector and that are necessary for the formation of the viral particles.

[0118] The expression "S protein family" is understood here to mean the complete S protein, its ectodomain and fragments of this ectodomain which are preferably produced in a eukaryotic system.

[0119] The subject of the present invention is also a lentiviral vector encoding a polypeptide of the S protein family, as defined above.

[0120] The subject of the present invention is also a recombinant measles virus encoding a polypeptide of the S protein family, as defined above.

[0121] The subject of the present invention is also a recombinant vaccinia virus encoding a polypeptide of the S protein family, as defined above.

[0122] The subject of the present invention is also the use of a vector according to paragraphs e) to r) as defined above, or of a vector containing a synthetic gene for the S protein, as defined above, for the production, in a eukaryotic system, of the SARS-associated coronavirus S protein or of a fragment of this protein.

[0123] The subject of the present invention is also a method for producing the S protein in a eukaryotic system, comprising a step of transfecting eukaryotic cells in culture with a vector chosen from the vectors contained in the bacterial strains mentioned in paragraphs e) to r) above or a vector containing a synthetic gene allowing optimized expression of the S protein.

[0124] The subject of the present invention is also a cDNA library characterized in that it comprises fragments as defined above, in particular amplification fragments or restriction fragments, cloned into a recombinant vector, in particular an expression vector (expression library).

[0125] The subject of the present invention is also cells, in particular prokaryotic cells, modified by a recombinant vector as defined above.

[0126] The subject of the present invention is also a genetically modified eukaryotic cell expressing a protein or a polypeptide as defined above. Quite obviously, the terms "genetically modified eukaryotic cell" do not denote a cell modified with a wild-type virus.

[0127] According to an advantageous embodiment of said cell, it is capable of being obtained by transfection with any of the vectors mentioned in paragraphs K) to N) above.

[0128] According to an advantageous feature of this embodiment, this is the cell FRhK4-Ssol-30, deposited at the CNCM on Nov. 22, 2004, under the No. I-3325.

[0129] The recombinant vectors as defined above and the cells transformed with said expression vectors are advantageously used for the production of the corresponding proteins and peptides. The expression libraries derived from

said vectors, and the cells transformed with said expression libraries are advantageously used to identify the immunogenic epitopes (B and T epitopes) of the SARS-associated coronavirus proteins.

[0130] The subject of the present invention is also the purified or isolated proteins and peptides, characterized in that they are encoded by the polynucleotide or one of its fragments as defined above.

[0131] According to an advantageous embodiment of the invention, said protein is selected from the group consisting of:

[0132] the S protein having the sequence SEQ ID NO: 3 or its ectodomain

[0133] the E protein having the sequence SEQ ID NO: 14

[0134] the M protein having the sequence SEQ ID NO: 17

[0135] the N protein having the sequence SEQ ID NO: 37

[0136] the proteins encoded by the ORFs: ORF1a, ORF1b, ORF3, ORF4 and ORF7 to ORF11, ORF13 and ORF14 and having the respective sequence, SEQ ID NO: 74, 75, 10, 12, 22, 24, 26, 28, 30, 33 and 35.

[0137] The terms "ectodomain of the S protein" and "soluble form of the S protein" will be used interchangeably below.

[0138] According to an advantageous embodiment of the invention, said polypeptide consists of the amino acids corresponding to positions 1 to 1193 of the amino acid sequence of the S protein.

[0139] According to another advantageous embodiment of the invention, said peptide is selected from the group consisting of:

[0140] a) the peptides corresponding to positions 14 to 1193 and 475 to 1193 of the amino acid sequence of the S protein,

[0141] b) the peptides corresponding to positions 2 to 14 (SEQ ID NO: 69) and 100 to 221 of the amino acid sequence of the M protein; these peptides correspond respectively to the ectodomain and to the endodomain of the M protein, and

[0142] c) the peptides corresponding to positions 1 to 12 (SEQ ID NO: 70) and 53 to 76 (SEQ ID NO: 71) of the amino acid sequence of the E protein; these peptides correspond respectively to the ectodomain and to the C-terminal end of the E protein, and

[0143] d) the peptides of 5 to 50 consecutive amino acids, preferably of 10 to 30 amino acids, inclusive or partially or completely overlapping the sequence of the peptides as defined in a), b) or c).

[0144] The subject of the present invention is also a peptide, characterized in that it has a sequence of 7 to 50 amino acids including an amino acid residue selected from the group consisting of:

[0145] the alanine situated at position 2552 of the amino acid sequence of the protein encoded by ORF1a,

[0146] the serine situated at position 577 of the amino acid sequence of the S protein of the SARS-CoV strain as defined above,

[0147] the glycine at position 11 of the amino acid sequence of the protein encoded by ORF3 of the SARS-CoV strain as defined above,

[0148] the serine at position 154 of the amino acid sequence of the M protein of the SARS-CoV strain as defined above.

[0149] The subject of the present invention is also an antibody or a polyclonal or monoclonal antibody fragment which can be obtained by immunization of an animal with a recombinant vector as defined above, a cDNA library as defined above or alternatively a protein or a peptide as defined above, characterized in that it binds to at least one of the proteins encoded by SARS-CoV as defined above.

[0150] The invention encompasses the polyclonal antibodies, the monoclonal antibodies, the chimeric antibodies such as the humanized antibodies, and fragments thereof (Fab, Fv, scFv).

[0151] A subject of the present invention is also a hybridoma producing a monoclonal antibody against the N protein, characterized in that it is chosen from the following hybridomas:

[0152] the hybridoma producing the monoclonal antibody 87, deposited at the CNCM on Dec. 1, 2004 under the number I-3328,

[0153] the hybridoma producing the monoclonal antibody 86, deposited at the CNCM on Dec. 1, 2004 under the number I-3329,

[0154] the hybridoma producing the monoclonal antibody 57, deposited at the CNCM on Dec. 1, 2004 under the number I-3330, and

[0155] the hybridoma producing the monoclonal antibody 156, deposited at the CNCM on Dec. 1, 2004 under the number I-3331.

[0156] The subject of the present invention is also a polyclonal or monoclonal antibody or antibody fragment directed against the N protein, characterized in that it is produced by a hybridoma as defined above.

[0157] For the purposes of the present invention, the expression chimeric antibody is understood to mean, in relation to an antibody of a particular animal species or of a particular class of antibody, an antibody comprising all or part of a heavy chain and/or of a light chain of an antibody of another animal species or of another class of antibody.

[0158] For the purposes of the present invention, the expression humanized antibody is understood to mean a human immunoglobulin in which the residues of the CDRs (Complementary Determining Regions) which form the antigen-binding site are replaced by those of a nonhuman monoclonal antibody possessing the desired specificity, affinity or activity. Compared with the nonhuman antibodies, the humanized antibodies are less immunogenic and possess a prolonged half-life in humans because they possess only a small proportion of nonhuman sequences given that practically all the residues of the FR (Framework) regions and of

the constant (Fc) region of these antibodies are those of a consensus sequence of human immunoglobulins.

[0159] A subject of the present invention is also a protein chip or filter, characterized in that it comprises a protein, a peptide or alternatively an antibody as defined above.

[0160] The protein chips according to the invention are prepared by conventional methods known per se. Among the appropriate supports on which proteins may be immobilized, there may be mentioned those made of plastic or glass, in particular in the form of microplates.

[0161] The subject of the present invention is also reagents derived from the isolated strain of SARS-associated coronavirus, derived from the sample recorded under the No. 031589, which are useful for the study and diagnosis of the infection caused by a SARS-associated coronavirus, said reagents are selected from the group consisting of:

[0162] (a) a pair of primers, a probe or a DNA chip as defined above,

[0163] (b) a recombinant vector or a modified cell as defined above,

[0164] (c) an isolated coronavirus strain or a polynucleotide as defined above,

[0165] (d) a protein or a peptide as defined above,

[0166] (e) an antibody or an antibody fragment as defined above, and

[0167] (f) a protein chip as defined above.

[0168] These various reagents are prepared and used according to conventional molecular biology and immunology techniques following standard protocols such as those described in *Current Protocols in Molecular Biology* (Frederick M. AUSUBEL, 2000, Wiley and Son Inc., Library of Congress, USA), in *Current Protocols in Immunology* (John E. Cologan, 2000, Wiley and Son Inc., Library of Congress, USA) and in *Antibodies: A Laboratory Manual* (E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

[0169] The nucleic acid fragments according to the invention are prepared and used according to conventional techniques as defined above. The peptides and proteins according to the invention are prepared by recombinant DNA techniques, known to persons skilled in the art, in particular with the aid of the recombinant vectors as defined above. Alternatively, the peptides according to the invention may be prepared by conventional techniques of solid or liquid phase synthesis, known to persons skilled in the art.

[0170] The polyclonal antibodies are prepared by immunizing an appropriate animal with a protein or a peptide as defined above, optionally coupled to KLH or to albumin and/or combined with an appropriate adjuvant such as (complete or incomplete) Freund's adjuvant or aluminum hydroxide; after obtaining a satisfactory antibody titer, the antibodies are harvested by collecting serum from the immunized animals and enriched with IgG by precipitation, according to conventional techniques, and then the IgGs specific for the SARS-CoV proteins are optionally purified by affinity chromatography on an appropriate column to which said peptide or said protein is attached, as defined above, so as to obtain a monospecific IgG preparation.

[0171] The monoclonal antibodies are produced from hybridomas obtained by fusion of B lymphocytes from an animal immunized with a protein or a peptide as defined above with myelomas, according to the Kohler and Milstein technique (Nature, 1975, 256, 495-497); the hybridomas are cultured in vitro, in particular in fermenters or produced in vivo, in the form of ascites; alternatively, said monoclonal antibodies are produced by genetic engineering as described in American patent U.S. Pat. No. 4,816,567.

[0172] The humanized antibodies are produced by general methods such as those described in International application WO 98/45332.

[0173] The antibody fragments are produced from the cloned V_H and V_L regions, from the mRNAs of hybridomas or splenic lymphocytes of an immunized mouse; for example, the Fv, scFv or Fab fragments are expressed at the surface of filamentous phages according to the Winter and Milstein technique (Nature, 1991, 349, 293-299); after several selection steps, the antibody fragments specific for the antigen are isolated and expressed in an appropriate expression system, by conventional techniques for cloning and expression of recombinant DNA.

[0174] The antibodies or fragments thereof as defined above are purified by conventional techniques known to persons skilled in the art, such as affinity chromatography.

[0175] The subject of the present invention is additionally the use of a product selected from the group consisting of: a pair of primers, a probe, a DNA chip, a recombinant vector, a modified cell, an isolated coronavirus strain, a polynucleotide, a protein or a peptide, an antibody or an antibody fragment and a protein chip as defined above, for the preparation of a reagent for the detection and optionally genotyping/serotyping of a SARS-associated coronavirus.

[0176] The proteins and peptides according to the invention, which are capable of being recognized and/or of inducing the production of antibodies specific for the SARS-associated coronavirus, are useful for the diagnosis of infection with such a coronavirus; the infection is detected, by an appropriate technique—in particular EIA, ELISA, RIA, immunofluorescence—, in a biological sample collected from an individual capable of being infected.

[0177] According to an advantageous feature of said use, said proteins are selected from the group consisting of the S, E, M and/or N proteins and the peptides as defined above.

[0178] The S, E, M and/or N proteins and the peptides derived from these proteins as defined above, for example the N protein, are used for the indirect diagnosis of a SARS-associated coronavirus infection (serological diagnosis; detection of an antibody specific for SARS-CoV), in particular by an immunoenzymatic method (ELISA).

[0179] The antibodies and antibody fragments according to the invention, in particular those directed against the S, E, M and/or N proteins and the derived peptides as defined above, are useful for the direct diagnosis of a SARS-associated coronavirus infection; the detection of the protein(s) of SARS-CoV is carried out by an appropriate technique, in particular EIA, ELISA, RIA, immunofluorescence, in a biological sample collected from an individual capable of being infected.

[0180] The subject of the present invention is also a method for the detection of a SARS-associated coronavirus, from a biological sample, which method is characterized in that it comprises at least:

[0181] (a) bringing said biological sample into contact with at least one antibody or one antibody fragment, one protein, one peptide or alternatively one protein or peptide chip or filter as defined above, and

[0182] (b) visualizing by any appropriate means antigen-antibody complexes formed in (a), for example by EIA, ELISA, RIA, or by immunofluorescence.

[0183] According to one advantageous embodiment of said process, step (a) comprises:

[0184] (a_1) bringing said biological sample into contact with at least a first antibody or an antibody fragment which is attached to an appropriate support, in particular a microplate,

[0185] (a_2) washing the solid phase, and

[0186] (a_3) adding at least a second antibody or an antibody fragment, different from the first, said antibody or antibody fragment being optionally appropriately labeled.

[0187] This method, which makes it possible to capture the viral particles present in the biological sample, is also called immunocapture method.

[0188] For example:

[0189] step (a_1) is carried out with at least a first monoclonal or polyclonal antibody or a fragment thereof, directed against the S, M and/or E protein, and/or a peptide corresponding to the ectodomain of one of these proteins (M2-14 or E1-12 peptides)

[0190] step (a_3) is carried out with at least one antibody or an antibody fragment directed against another epitope of the same protein or preferably against another protein, preferably against an inner protein such as the N nucleoprotein or the endodomain of the E or M protein, more preferably still these are antibodies or antibody fragments directed against the N protein which is very abundant in the viral particle; when an antibody or an antibody fragment directed against an inner protein (N) or against the endodomain of the E or M proteins is used, said antibody is incubated in the presence of detergent, such as Tween 20 for example, at concentrations of the order of 0.1%.

[0191] step (b) for visualizing the antigen-antibody complexes formed is carried out, either directly with the aid of a second antibody labeled for example with biotin or an appropriate enzyme such as peroxidase or alkaline phosphatase, or indirectly with the aid of an anti-immunoglobulin serum labeled as above. The complexes thus formed are visualized with the aid of an appropriate substrate.

[0192] According to a preferred embodiment of this aspect of the invention, the biological sample is mixed with the visualizing monoclonal antibody prior to its being brought into contact with the capture monoclonal antibodies. Where appropriate, the serum-visualizing antibody mixture is incubated for at least 10 minutes at room temperature before being applied to the plate.

[0193] The subject of the present invention is also an immunocapture test intended to detect an infection by the SARS-associated coronavirus by detecting the native nucleoprotein (N protein), in particular characterized in that the antibody used for the capture of the native viral nucleoprotein is a monoclonal antibody specific for the central region and/or for a conformational epitope.

[0194] According to one embodiment of said test, the antibody used for the capture of the N protein is the monoclonal antibody mAb87, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3328.

[0195] According to another embodiment of said immunocapture test, the antibody used for the capture of the N protein is the monoclonal antibody mAb86, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3329.

[0196] According to another embodiment of said immunocapture test, the monoclonal antibodies mAb86 and mAb87 are used for the capture of the N protein.

[0197] In the immunocapture tests according to the invention, it is possible to use, for visualizing the N protein, the monoclonal antibody mAb57, produced by the hybridoma deposited at the CNCM on Dec. 1, 2004 under the number I-3330, said antibody being conjugated with a visualizing molecule or particle.

[0198] In accordance with said immunocapture test, a combination of the antibodies mAb57 and mAb87, conjugated with a visualizing molecule or particle, is used for the visualization of the N protein.

[0199] A visualizing molecule may be a radioactive atom, a dye, a fluorescent molecule, a fluorophore, an enzyme; a visualizing particle may be for example: colloidal gold, a magnetic particle or a latex bead.

[0200] The subject of the present invention is also a reagent for detecting a SARS-associated coronavirus, characterized in that it is selected from the group consisting of:

[0201] (a) a pair of primers or a probe as defined above,

[0202] (b) a recombinant vector as defined above or a modified cell as defined above,

[0203] (c) an isolated coronavirus strain as defined above or a polynucleotide as defined above,

[0204] (d) an antibody or an antibody fragment as defined above,

[0205] (e) a combination of antibodies comprising the monoclonal antibodies mAb86 and/or mAb87, and the monoclonal antibody mAb57, as defined above,

[0206] (f) a chip or a filter as defined above.

[0207] The subject of the present invention is also a method for the detection of a SARS-associated coronavirus infection, from a biological sample, by indirect IgG ELISA using the N protein, which method is characterized in that the plates are sensitized with an N protein solution at a concentration of between 0.5 and 4 μg/ml, preferably to 2 μg/ml, in a 10 mM PBS buffer pH 7.2, phenol red at 0.25 ml/l.

[0208] The subject of the present invention is additionally a method for the detection of a SARS-associated coronavirus infection, from a biological sample, by double epitope ELSA, characterized in that the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.

[0209] According to one variant of the tests for detecting SARS-associated coronaviruses, these tests combine an ELSA using the N protein, and another ELSA using the S protein, as described below.

[0210] The subject of the present invention is also an immune complex formed of a polyclonal or monoclonal antibody or antibody fragment as defined above, and of a SARS-associated coronavirus protein or peptide.

[0211] The subject of the present invention is additionally a SARS-associated coronavirus detection kit, characterized in that it comprises at least one reagent selected from the group consisting of: a pair of primers, a probe, a DNA or RNA chip, a recombinant vector, a modified cell, an isolated coronavirus strain, a polynucleotide, a protein or a peptide, an antibody, and a protein chip as defined above.

[0212] The subject of the present invention is additionally an immunogenic composition, characterized in that it comprises at least one product selected from the group consisting of:

[0213] a) a protein or a peptide as defined above,

[0214] b) a polynucleotide of the DNA or RNA type or one of its representative fragments as defined above, having a sequence chosen from:

[0215] (i) the sequence SEQ ID NO: 1 or its RNA equivalent

[0216] (ii) the sequence hybridizing under high stringency conditions with the sequence SEQ ID NO: 1,

[0217] (iii) the sequence complementary to the sequence SEQ ID NO: 1 or to the sequence hybridizing under high stringency conditions with the sequence SEQ ID NO: 1,

[0218] (iv) the nucleotide sequence of a representative fragment of the polynucleotide as defined in (i), (ii) or (iii),

[0219] (v) the sequence as defined in (i), (ii), (iii) or (iv), modified, and

[0220] c) a recombinant expression vector comprising a polynucleotide as defined in b), and

[0221] d) a cDNA library as defined above,

said immunogenic composition being capable of inducing protective humoral or cellular immunity specific for the SARS-associated coronavirus, in particular the production of an antibody directed against a specific epitope of the SARS-associated coronavirus.

[0222] The proteins and peptides as defined above, in particular the S, M, E and/or N proteins and the derived peptides, and the nucleic acid (DNA or RNA) molecules encoding said proteins or said peptides are good candidate vaccines and may be used in immunogenic compositions for the production of a vaccine against the SARS-associated coronavirus.

[0223] According to an advantageous embodiment of the compositions according to the invention, they additionally contain at least one pharmaceutically acceptable vehicle and optionally carrier substances and/or adjuvants.

[0224] The pharmaceutically acceptable vehicles, the carrier substances and the adjuvants are those conventionally used.

[0225] The adjuvants are advantageously chosen from the group consisting of oily emulsions, saponin, mineral substances, bacterial extracts, aluminum hydroxide and squalene.

[0226] The carrier substances are advantageously selected from the group consisting of unilamellar liposomes, multilamellar liposomes, micelles of saponin or solid microspheres of a saccharide or auriferous nature.

[0227] The compositions according to the invention are administered by the general route, in particular by the intramuscular or subcutaneous route or alternatively by the local, in particular nasal (aerosol) route.

[0228] The subject of the present invention is also the use of an isolated or purified protein or peptide having a sequence selected from the group consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75 to form an immune complex with an antibody specifically directed against an epitope of the SARS-associated coronavirus.

[0229] The subject of the present invention is also an immune complex consisting of an isolated or purified protein or peptide having a sequence selected from the group consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75, and of an antibody specifically directed against an epitope of the SARS-associated coronavirus.

[0230] The subject of the present invention is also the use of an isolated or purified protein or peptide having a sequence selected from the group consisting of the sequences SEQ ID NO: 3, 10, 12, 14, 17, 22, 24, 26, 28, 30, 33, 35, 37, 69, 70, 71, 74 and 75 to induce the production of an antibody capable of specifically recognizing an epitope of the SARS-associated coronavirus.

[0231] The subject of the present invention is also the use of an isolated or purified polynucleotide having a sequence selected from the group consisting of the sequences SEQ ID NO: 1, 2, 4, 7, 8, 13, 15, 16, 18, 19, 20, 31, 36 and 38 to induce the production of an antibody directed against the protein encoded by said polynucleotide and capable of specifically recognizing an epitope of the SARS-associated coronavirus.

[0232] The subject of the present invention is also monoclonal antibodies recognizing the native S protein of a SARS-associated coronavirus.

[0233] The subject of the present invention is also the use of a protein or a polypeptide of the S protein family, as defined above, or of an antibody recognizing the native S protein, as defined above, to detect an infection by a SARS-associated coronavirus, in a biological sample.

[0234] The subject of the present invention is also a method for detecting an infection by a SARS-associated coronavirus, in a biological sample, characterized in that the

detection is carried out by ELISA using the recombinant S protein, expressed in a eukaryotic system.

[0235] According to an advantageous embodiment of said method, it is a double epitope ELISA method, and the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.

[0236] The subject of the present invention is also an immune complex consisting of a monoclonal antibody or antibody fragment recognizing the native S protein, and of a protein or a peptide of the SARS-associated coronavirus.

[0237] The subject of the present invention is also an immune complex consisting of a protein or a polypeptide of the S protein family, as defined above, and of an antibody specifically directed against an epitope of the SARS-associated coronavirus.

[0238] The subject of the present invention is additionally a SARS-associated coronavirus detection kit or box, characterized in that it comprises at least one reagent selected from the group consisting of: a protein or polypeptide of the S protein family, as defined above, a nucleic acid encoding a protein or peptide of the S protein family, as defined above, a cell expressing a protein or polypeptide of the S protein family, as defined above, or an antibody recognizing the native S protein of a SARS-associated coronavirus.

[0239] The subject of the present invention is an immunogenic and/or vaccine composition, characterized in that it comprises a polypeptide or a recombinant protein of the S protein family, as defined above, obtained in a eukaryotic expression system.

[0240] The subject of the present invention is also an immunogenic and/or vaccine composition, characterized in that it comprises a vector or recombinant virus, expressing a protein or a polypeptide of the S protein family, as defined above.

[0241] In addition to the preceding features, the invention further comprises other features, which will emerge from the description which follows, which refers to examples of use of the polynucleotide representing the genome of the SARS-CoV strain derived from the sample recorded under the number 031589, and derived cDNA fragments which are the subject of the present invention, and to Table I presenting the sequence listing:

TABLE I

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid
SEQ ID NO: 1	genome of the strain derived from the sample 031589	—	—
SEQ ID NO: 2	ORF-S*	21406-25348	—
SEQ ID NO: 3	S protein	—	—
SEQ ID NO: 4	ORF-S**	21406-25348	I-3059

TABLE I-continued

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the corresponding plasmid
SEQ ID NO: 5	Sa fragment	21406-23454	I-3020
SEQ ID NO: 6	Sb fragment	23322-25348	I-3019
SEQ ID NO: 7	ORF-3 + ORF-4*	25110-26244	—
SEQ ID NO: 8	ORF-3 + ORF-4**	25110-26244	I-3126
SEQ ID NO: 9	ORF3	—	—
SEQ ID NO: 10	ORF-3 protein	—	—
SEQ ID NO: 11	ORF4	—	—
SEQ ID NO: 12	ORF-4 protein	—	—
SEQ ID NO: 13	ORF-E*	26082-26413	—
SEQ ID NO: 14	E protein	—	—
SEQ ID NO: 15	ORF-E**	26082-26413	I-3046
SEQ ID NO: 16	ORF-M*	26330-27098	—
SEQ ID NO: 17	M protein	—	—
SEQ ID NO: 18	ORF-M**	26330-27098	I-3047
SEQ ID NO: 19	ORF7 to 11*	26977-28218	—
SEQ ID NO: 20	ORF7 to 11**	26977-28218	I-3125
SEQ ID NO: 21	ORF7	—	—
SEQ ID NO: 22	ORF7 protein	—	—
SEQ ID NO: 23	ORF8	—	—
SEQ ID NO: 24	ORF8 protein	—	—
SEQ ID NO: 25	ORF9	—	—
SEQ ID NO: 26	ORF9 protein	—	—
SEQ ID NO: 27	ORF10	—	—
SEQ ID NO: 28	ORF10 protein	—	—
SEQ ID NO: 29	ORF11	—	—
SEQ ID NO: 30	ORF11 protein	—	—
SEQ ID NO: 31	OrF1ab	265-21485	—
SEQ ID NO: 32	ORF13	28130-28426	—
SEQ ID NO: 33	ORF13 protein	—	—
SEQ ID NO: 34	ORF14	—	—
SEQ ID NO: 35	ORF14 protein	28583-28795	—
SEQ ID NO: 36	ORF-N*	28054-29430	
SEQ ID NO: 37	N protein	—	—
SEQ ID NO: 38	ORF-N**	28054-29430	I-3048
SEQ ID NO: 39	noncoding 5'**	1-204	I-3124
SEQ ID NO: 40	noncoding 3'**	28933-29727	I-3123
SEQ ID NO: 41	ORF1ab Fragment L0	30-500	—
SEQ ID NO: 42	Fragment L1	211-2260	—
SEQ ID NO: 43	Fragment L2	2136-4187	—
SEQ ID NO: 44	Fragment L3	3892-5344	—
SEQ ID NO: 45	Fragment L4b	4932-6043	—
SEQ ID NO: 46	Fragment L4	5305-7318	—
SEQ ID NO: 47	Fragment L5	7275-9176	—
SEQ ID NO: 48	Fragment L6	9032-11086	—
SEQ ID NO: 49	Fragment L7	10298-10982	—
SEQ ID NO: 50	Fragment L8	12815-14854	—
SEQ ID NO: 51	Fragment L9	14745-16646	—
SEQ ID NO: 52	Fragment L10	16514-18590	—
SEQ ID NO: 53	Fragment L11	18500-20602	—
SEQ ID NO: 54	Fragment L12	20319-22224	—
SEQ ID NO: 55	Sense N primer	—	—
SEQ ID NO: 56	Antisense N primer	—	—
SEQ ID NO: 57	Sense S_C primer	—	—
SEQ ID NO: 58	Sense S_L primer	—	—
SEQ ID NO: 59	Antisense S_C and S_L primer	—	—
SEQ ID NO: 60	Sense primer series 1	28507-28522	—
SEQ ID NO: 61	Antisense primer series 1	28774-28759	
SEQ ID NO: 62	Sense primer series 2	28375-28390	—
SEQ ID NO: 63	Antisense primer series 2	28702-28687	—

TABLE I-continued

Sequence listing

Identification number	Sequence	Position of the cDNA with reference to Genbank AY274119.3	Deposit number at the CNCM of the correspond-ing plasmid
SEQ ID NO: 64	Probe 1/series 1	28561-28586	—
SEQ ID NO: 65	Probe 2/series 1	28588-28608	—
SEQ ID NO: 66	Probe 1/series 2	28541-28563	—
SEQ ID NO: 67	Probe 2/series 2	28565-28589	—
SEQ ID NO: 68	Anchor primer 14T		
SEQ ID NO: 69	Peptide M2-14	—	—
SEQ ID NO: 70	Peptide E1-12	—	—
SEQ ID NO: 71	Peptide E53-76	—	—
SEQ ID NO: 72	Noncoding 5'*	1-204	—
SEQ ID NO: 73	Noncoding 3'*	28933-29727	—
SEQ ID NO: 74	ORF1a protein	—	—
SEQ ID NO: 75	ORF1b protein	—	—
SEQ ID NO: 76-139	Primers		
SEQ ID NO: 140	Pseudogene of S		
SEQ ID NO: 141-148	Primers		
SEQ ID NO: 149	Aa1-13 of S		
SEQ ID NO: 150	Polypeptide		
SEQ ID NO: 151-158	Primers		

*PCR amplification product (amplicon)

**Insert cloned into the plasmid deposited at the CNCM and to the appended drawings in which:

[0242] FIG. 1 illustrates Western-blot analysis of the expression in vitro of the recombinant proteins N, S_C and S_L from the expression vectors pIVEX. Lane 1: pIV2.3N. Lane 2: pIV2.3S_C. Lane 3: pIV2.3S_L. Lane 4: pIV2.4N. Lane 5: pIV2.4S_1 or pIV2.4S_C. Lane 6: pIV2.4S_L. The expression of the GFP protein expressed from the same vector is used as a control.

[0243] FIG. 2 illustrates the analysis, by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and staining with Coomassie blue, of the expression in vivo of the N protein from the expression vectors pIVEX. The *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX is cultured at 30° C. in LB medium, in the presence or in the absence of inducer (IPTG 1 mM). Lane 1: pIV2.3N. Lane 2: pIV2.4N.

[0244] FIG. 3 illustrates the analysis, by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE) and staining with Coomassie blue, of the expression in vivo of the S_L and S_C polypeptides from the expression vectors pIVEX. The *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX is cultured at 30° C. in LB medium, in the presence or in the absence of inducer (IPTG 1 mM). Lane 1: pIV2.3S_C. Lane 2: pIV2.3S_L. Lane 3: pIV2.4S_1. Lane 4: pIV2.4S_L.

[0245] FIG. 4 illustrates the antigenic activity of the recombinant N, S_L and S_C proteins produced in the *E. coli* BL21(DE3)pDIA17 strain transformed with the recombinant vectors pIVEX. A: electrophoresis (SDS-PAGE) of the bacterial lysates. B and C: Western-blot with the sera, obtained from the same patient infected with SARS-CoV, collected 8 days (B: serum M12) and 29 days (C: serum M13) respectively after the onset of the SARS symptoms. Lane 1: pIV2.3N. Lane 2: pIV2.4N. Lane 3: pIV2.3S_C. Lane 4: pIV2.4S_1. Lane 5: pIV2.3S_L. Lane 6: pIV2.4S_L.

[0246] FIG. 5 illustrates the purification on an Ni-NTA agarose column of the recombinant N protein produced in the *E. coli* BL21(DE3)pDIA17 strain from the vector pIV2.3N. Lane 1: total bacterial extract. Lane 2: soluble extract. Lane 3: insoluble extract. Lane 4: extract deposited on the Ni-NTA column. Lane 5: unbound proteins. Lane 6: fractions of peak 1. Lane 7: fractions of peak 2.

[0247] FIG. 6 illustrates the purification of the recombinant S_C protein from the inclusion bodies produced in the *E. coli* BL21(DE3)pDIA17 strain transformed with pIV2.4S_1. A. Treatment with Triton X-100 (2%): Lane 1: total bacterial extract. Lane 2: soluble extract. Lane 3: insoluble extract. Lane 4: supernatant after treatment with Triton X-100 (2%). Lanes 5 and 6: pellet after treatment with Triton X-100 (2%). B: Treatment with 4 M, 5 M, 6 M and 7 M urea of the soluble and insoluble extracts.

[0248] FIG. 7 represents the immunoblot produced with the aid of a lysate of cells infected with SARS-CoV and a serum from a patient suffering from atypical pneumopathy.

[0249] FIG. 8 represents immunoblots produced with the aid of a lysate of cells infected with SARS-CoV and rabbit immunosera specific for the nucleoprotein N (A) and for the spicule protein S (B). I.S.: immune serum. p.i.: preimmune serum. The anti-N immune serum was used at 1/50 000 and the anti-S immune serum at 1/10 000.

[0250] FIG. 9 illustrates the ELISA reactivity of the rabbit monospecific polyclonal sera directed against the N protein or the short fragment of the S protein (S_C), toward the corresponding recombinant proteins used for immunization. A: rabbits P13097, P13081 and P13031 immunized with the purified recombinant N protein. B: rabbits P11135, P13042 and P14001 immunized with a preparation of inclusion bodies corresponding to the short fragment of the S protein (S_C). I.S.: immune serum. p.i.: preimmune serum.

[0251] FIG. 10 illustrates the ELISA reactivity of the purified recombinant N protein, toward sera from patients suffering from atypical pneumonia caused by SARS-CoV. FIG. 10*a*: ELISA plates prepared with the N protein at the concentration of 4 μg/ml and 2 μg/ml. FIG. 10B: ELISA plate prepared with the N protein at the concentration of 1 μg/ml. The sera designated A, B, D, E, F, G, H correspond to those of Table IV.

[0252] FIG. 11 illustrates the amplification by RT-PCR of decreasing quantities of synthetic RNA of the SARS-CoV N gene (10^7 to 1 copy), with the aid of pairs of primers No. 1 (N/+/28507, N/−/28774) (A) and No. 2 (N/+/28375, N/−/28702) (B). T: amplification performed in the absence of RNA. MW: DNA marker.

[0253] FIG. 12 illustrates the amplification by RT-PCR in real time of synthetic RNA for the SARS-CoV N gene: decreasing quantities of synthetic RNA as replica (repli.; lanes 16 to 29) and of viral RNA diluted $1/20 \times 10^{-4}$ (lane 32) were amplified by RT-PCR in real time with the aid of the kit "Light Cycler RNA Amplification Kit Hybridization Probes" and pairs of primers and probes of the No. 2 series, under the conditions described in Example 8.

[0254] FIG. 13 (FIGS. 13.1 to 13.7) represents the restriction map of the sequence SEQ ID NO: 1 corresponding to

the DNA equivalent of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589.

[0255] FIG. **14** shows the result of the SARS serology test by indirect N ELISA (1st series of sera tested).

[0256] FIG. **15** shows the result of the SARS serology test by indirect N ELISA (2nd series of sera tested).

[0257] FIG. **16** presents the result of the SARS serology test by double epitope N ELISA (1st series of sera tested).

[0258] FIG. **17** shows the result of the SARS serology test by double epitope N ELISA (2nd series of sera tested).

[0259] FIG. **18** illustrates the test of reactivity of the anti-N monoclonal antibodies by ELISA on the native nucleoprotein N of SARS-CoV. The antibodies were tested in the form of hybridoma culture supernatants by indirect ELISA using an irradiated lysate of VeroE6 cells infected with SARS-CoV as antigen (SARS lysate curves). A negative control for reactivity is performed for each antibody on a lysate of uninfected VeroE6 cells (negative lysate curves). Several monoclonal antibodies of known specificity were used as negative control antibodies: para1-3 directed against the antigens of the parainfluenza viruses type 1-3 (Bio-Rad) and influenza B directed against the antigens of the influenza virus type B (Bio-Rad).

[0260] FIG. **19** illustrates the test of reactivity of the anti-N of SARS-CoV monoclonal antibodies by ELISA on the native antigens of the human coronavirus 229E (HCoV-229E). The antibodies were tested in the form of hybridoma culture supernatants by an indirect ELISA test using a lysate of MRC-5 cells infected with the human coronavirus 229E as antigen (229E lysate curves). A negative control for immunoreactivity was performed for each antibody on a lysate of noninfected MRC-5 cells (negative lysate curves). The monoclonal antibody 5-11H.6 directed against the S protein of the human coronavirus 229E (Sizun et al. 1998, J. Virol. Met. 72: 145-152) is used as positive control antibody. The antibodies para1-3 directed against the antigens of the parainfluenza virus type 1-3 (Bio-Rad) and influenza B directed against the antigens of the influenza virus type B (Bio-Rad) were added to the panel of monoclonal antibodies tested.

[0261] FIG. **20** shows a test of reactivity of the anti-N of SARS-CoV monoclonal antibodies by Western blotting on the denatured native nucleoprotein N of SARS-CoV. A lysate of VeroE6 cells infected with SARS-CoV was prepared in the loading buffer according to Laemmli and caused to migrate in a 12% SDS polyacrylamide gel and then the proteins were transferred onto PVDF membrane. The anti-N monoclonal antibodies tested were used for the immunoassay at the concentration of 0.05 μg/ml. The visualization is carried out with anti-mouse IgG(H+L) antibodies coupled to peroxidase (NA931V, Amersham) and the ECL+ system. Two monoclonal antibodies were used as negative controls for reactivity: influenza B directed against the antigens of the influenza virus type B (Bio-Rad) and para1-3 directed against the antigens of the parainfluenza virus type 1-3 (Bio-Rad).

[0262] FIG. **21** presents the plasmids for expression in mammalian cells of the SARS-CoV S protein. The cDNA for the SARS-CoV S was inserted between the BamHI and Xho1 sites of the expression plasmid pcDNA3.1(+) (Clontech) in order to obtain the plasmid pcDNA-S and between the Nhe1 and Xho1 sites of the expression plasmid pCI (Promega) in order to obtain the plasmid pCI-S. The WPRE and CTE sequences were inserted between each of the two plasmids pcDNA-S and pCI-S between the Xho1 and Xba1 sites in order to obtain the plasmids pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S-CTE and pCI-S-WPRE, respectively.

[0263] SP: signal peptide predicted (aa 1-13) with the software signalP v2.0 (Nielsen et al., 1997, Protein Engineering, 10:1-6)

[0264] TM: transmembrane region predicted (aa 1196-1218) with the software TMHMM v2.0 (Sonnhammer et al., 1998, Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, pp. 175-182, AAAI Press). It should be noted that the amino acids W1194 and P1195 are possibly part of the transmembrane region with the respective probabilities of 0.13 and 0.42

[0265] P-CMV: cytomegalovirus immediate/early promoter. BGH pA: polyadenylation signal of the bovine growth hormone gene

[0266] SV40 late pA: SV40 virus late polyadenylation signal

[0267] SD/SA: splice donor and acceptor sites

[0268] WPRE: sequences of the "Woodchuck Hepatitis Virus posttranscriptional regulatory element" of the woodchuck hepatitis virus

[0269] CTE: sequences of the "constitutive transport element" of the Mason-Pfizer simian retrovirus

[0270] FIG. **22** illustrates the expression of the S protein after transfection of VeroE6 cells. Cellular extracts were prepared 48 hours after transfection of VeroE6 cells with the plasmids pcDNA, pcDNA-S, pCI and pCI-S. Cellular extracts were also prepared 18 hours after infection with the recombinant vaccinia virus VV-TF7.3 and transfection with the plasmids pcDNA or pcDNA-S. As a control, extracts of VeroE6 cells were prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 3. They were separated on an 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). A molecular mass ladder (kDa) is presented in the figure.

[0271] SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

[0272] Mock: control extract of noninfected cells

[0273] FIG. **23** illustrates the effect of the CTE and WPRE sequences on the expression of the S protein after transfection of VeroE6 and 293T cells. Cellular extracts were prepared 48 hours after transfection of VeroE6 cells (A) or 293T cells (B) with the plasmids pcDNA, pcDNA-S, pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S, pCI-S-CTE and pCI-S-WPRE separated on 8% SDS polyacrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). A molecular mass ladder (kDa) is presented in the figure.

[0274] SARS-CoV: extract of VeroE6 cells prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 3.

[0275] Mock: control extract of noninfected VeroE6 cells

[0276] FIG. **24** presents defective lentiviral vectors with central DNA flap for the expression of SARS-CoV S. The cDNA for the SARS-CoV S protein was cloned in the form of a BamH1-Xho1 fragment into the plasmid pTRIPΔU3-CMV containing a defective lentiviral vector TRIP with central DNA flap (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmid pTRIP-S. The optimum expression cassettes consisting of the CMV virus immediate/early promoter, a splice signal, cDNA for S and either of the posttranscriptional signals CTE or WPRE were substituted for the cassette EF1α-EGFP of the defective lentiviral expression vector with central DNA flap TRIPΔU3-EF1α (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmids pTRIP-SD/SA-S-CTE and pTRIP-SD/SA-S-WPRE.

[0277] SP: signal peptide

[0278] TM: transmembrane region

[0279] P-CMV: cytomegalovirus immediate/early promoter

[0280] P-EF1α: EF1α gene promoter

[0281] SD/SA: splice donor and acceptor sites

[0282] WPRE: sequences of the "Woodchuck Hepatitis Virus posttranscriptional regulatory element" of the woodchuck hepatitis virus

[0283] CTE: sequences of the "constitutive transport element" of the Mason-Pfizer simian retrovirus

[0284] LTR: long terminal repeat

[0285] ΔU3: LTR deleted for the "promoter/enhancer" sequences

[0286] cPPT: "polypurine tract cis-active sequence"

[0287] CTS: "central termination sequence"

[0288] FIG. **25** shows the Western-blot analysis of the expression of the SARS-CoV S by cell lines transduced with the lentiviral vectors TRIP-SD/SA-S-WPRE and TRIP-SD/SA-S-CTE. Cellular extracts were prepared from established lines FrhK4-S-CTE and FrhK4-S-WPRE after transduction with the lentiviral vectors TRIP-SD/SA-S-CTE and TRIP-SD/SA-S-WPRE respectively. They were separated on an 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) conjugate coupled to peroxidase. A molecular mass ladder (kDa) is presented in the figure.

[0289] T−: control extract of FrhK-4 cells

[0290] T+: extract of FrhK-4 cells prepared 24 hours after infection with SARS-CoV at a multiplicity of infection of 3.

[0291] FIG. **26** relates to the analysis of the expression of Ssol polypeptide by cell lines transduced with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE. The secretion of the Ssol polypeptide was determined in the supernatant of a series of cell clones isolated after transduction of FrhK-4 cells with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE. 5 μl of supernatant, diluted ½ in loading buffer according to Laemmli, were analyzed by Western blotting, visualized with an anti-FLAG monoclonal antibody (M2, Sigma) and an anti-mouse IgG(H+L) conjugate coupled to peroxidase. T−: supernatant of the parental FRhK-4 line. T+: supernatant of BHK cells infected with a recombinant vaccinia virus expressing the Ssol polypeptide. The solid arrow indicates the Ssol polypeptide, while the empty arrow indicates a cross reaction with a protein of cellular origin.

[0292] FIG. **27** shows the results relating to the analysis of the purified Ssol polypeptide

[0293] A. 8, 2, 0.5 and 0.125 μg of recombinant Ssol polypeptide purified by anti-FLAG affinity chromatography and gel filtration (G75) were separated on 8% SDS polyacrylamide gel. The Ssol polypeptide and variable quantities of molecular mass markers (MM) were visualized by staining with silver nitrate (Gelcode SilverSNAP stain kit II, Pierce).

B. Standard markers for analysis by SELDI-TOF mass spectrometry

[0294] IgG: bovine IgG of MM 147300

[0295] ConA: conalbumin of MM 77490

[0296] HRP: horseradish peroxidase analyzed as a control and of MM 43240

C. Analysis by mass spectrometry (SELDI-TOF) of the recombinant Ssol polypeptide.

[0297] The peaks A and B correspond to the single and double charged Ssol polypeptide.

D. Sequencing of the N-terminal end of the recombinant Ssol polypeptide. 5 Edman degradation cycles in liquid phase were carried out on an ABI494 sequencer (Applied Biosystems).

[0298] FIG. **28** illustrates the influence of a splicing signal and of the CTE and WPRE sequences on the efficacy of the gene immunization with the aid of plasmid DNA encoding the SARS-CoV S

A. Groups of 7 BALB/c mice were immunized twice at 4 weeks' interval with the aid of 50 μg of plasmid DNA of pCI, pcDNA-S, pCI-S, pcDNA-N and pCI-HA.

B. Groups of 6 BALB/c mice were immunized twice at 4 weeks' interval with the aid of 2 μg, 10 μg or 50 μg of plasmid DNA of pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE.

[0299] The immune sera collected 3 weeks after the second immunization were analyzed by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (NA931V, Amersham) and TMB (KPL).

[0300] FIG. **29** shows the seroneutralization of the infectivity of SARS-CoV with the antibodies induced in mice after gene immunization with the aid of plasmid DNA encoding SARS-CoV S. Pools of immune sera collected 3 weeks after the second immunization were prepared for each

of the groups of experiments described in FIG. **28** and evaluated for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

A. Groups by BALB/c mice immunized twice at 4 weeks' interval with the aid of 50 μg of plasmid DNA of pCI, pcDNA-S, pCI-S, pcDNA-N and pCI-HA. □: preimmune serum. ■: immune serum.

B. Groups of BALB/c mice immunized twice at 4 weeks' interval with the aid of 2 μg, 10 μg or 50 μg of plasmid DNA of pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE.

[0301] FIG. **30** illustrates the immunoreactivity of the recombinant Ssol polypeptide toward sera from patients suffering from SARS. The reactivity of sera from patients was analyzed by indirect ELISA test against solid phases prepared with the aid of the purified recombinant Ssol polypeptide. The antibodies from patients reacting with the solid phase at a dilution of 1/400 are visualized with a human anti-IgG(H+L) polyclonal antibody coupled to peroxidase (Amersham NA933V) and TMB plus H202 (KPL). The sera of probable SARS cases are identified by a National Reference Center for Influenza Viruses serial number and by the initials of the patient and the number of days elapsed since the onset of symptoms, where appropriate. The TV sera are control sera from subjects which were collected in France before the SARS epidemic which occurred in 2003.

[0302] FIG. **31** shows the induction of antibodies directed against SARS-CoV after immunization with the recombinant Ssol polypeptide. Two groups of 6 mice were immunized at 3 weeks' interval with 10 μg of recombinant Ssol polypeptide (Ssol group) adjuvanted with aluminum hydroxide or, as a control, of adjuvant alone (mock group). Three successive immunizations were performed and the immune sera were collected 3 weeks after each of the three immunizations (IS1, IS2, IS3). The immune sera were analyzed per pool for each of the 2 groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (Amersham) and TMB (KPL).

[0303] FIG. **32** presents the nucleotide alignment of the sequences of the synthetic gene 040530 with the sequence of the wild-type gene of the SARS-CoV isolate 031589. I-3059 corresponds to nucleotides 21406-25348 of the SARS-CoV isolate 031589 deposited at the C.N.C.M. under the number I-3059 (SEQ ID NO: 4, plasmid pSARS-S)S-040530 is the sequence of the synthetic gene 040530.

[0304] FIG. **33** illustrates the use of a synthetic gene for the expression of the SARS-CoV S. Cellular extracts prepared 48 hours after transfection of VeroE6 cells (A) or 293T cells (B) with the plasmids pCI, pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-Ssynth were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (Fluor S, BioRad). The levels of expression of the S protein were measured by quantifying the 2 predominant bands identified on the image.

[0305] FIG. **34** presents a diagram for the construction of recombinant vaccinia viruses VV-TG-S, VV-TG-Ssol, VV-TN-S and W-TN-Ssol

A. The cDNAs for the S protein and the Ssol polypeptide of SARS-CoV were inserted between the BamH1 and SmaI sites of the transfer plasmid pTG186 in order to obtain the plasmids pTG-S and pTG-Ssol.

[0306] B. The sequences of the synthetic promoter 480 were then substituted for those of the 7.5 promoter by exchange of the NdeI-PstI fragments of the plasmids pTG186poly, pTG-S and pTG-Ssol in order to obtain the transfer plasmids pTN480, pTN-S and pTN-Ssol.

[0307] C. Sequence of the synthetic promoter 480 as contained between the NdeI and PstI sites of the transfer plasmids of the pTN series. An AscI site was inserted in order to facilitate subsequent handling. The restriction sites and the promoter sequence are underlined.

D. The recombinant vaccinia viruses are obtained by double homologous recombination in vivo between the TK cassette of the transfer plasmids of the pTG and pTN series and the TK gene of the Copenhagen strain of the vaccinia virus.

[0308] SP: signal peptide predicted (aa 1-13) with the software signalP v2.0 (Nielsen et al., 1997, Protein Engineering, 10:1-6)

[0309] TM: transmembrane region predicted (aa 1196-1218) with the software TMHMM v2.0 (Sonnhammer et al., 1998, Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, pp. 175-182, AAAI Press). It should be noted that the amino acids W1194 and P1195 possibly form part of the transmembrane region with respective probabilities of 0.13 and 0.42.

[0310] TK-L, TK-R: left- and right-hand parts of the vaccinia virus thymidine kinase gene

[0311] MCS: multiple cloning site

[0312] PE: early promoter

[0313] PL: late promoter

[0314] PL synth: synthetic late promoter 480

[0315] FIG. **35** illustrates the expression of the S protein by recombinant vaccinia viruses, analyzed by Western blotting. Cellular extracts were prepared 18 hours after infection of CV1 cells with the recombinant vaccinia viruses VV-TG, VV-TG-S and VV-TN-S at an M.O.I. of 2 (A). As a control, extracts of VeroE6 cells were prepared 8 hours after infection with SARS-CoV at a multiplicity of infection of 2. Cellular extracts were also prepared 18 hours after infection of CV1 cells with the recombinant vaccinia viruses VV-TG-S, VV-TG-Ssol, VV-TN, VV-TN-S and VV-TN-Ssol (B). They were separated on 8% SDS acrylamide gels and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham). "1 μl" and "10 μl" indicates the quantities of cellular extracts deposited on the gel. A molecular mass ladder (kDa) is presented in the figure.

[0316] SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

[0317] Mock: control extract of noninfected cells

[0318] FIG. 36 shows the result of a Western-blot analysis of the secretion of the Ssol polypeptide by the recombinant vaccinia viruses.

A. Supernatants of CV1 cells infected with the recombinant vaccinia virus VV-TN, various clones of the VV-TN-Ssol virus and with the viruses VV-TG-Ssol or VV-TN-Sflag were harvested 18 hours after infection of CV1 cells at an M.O.I. of 2.

[0319] B. Supernatants of 293T, FRhK-4, BHK-21 and CV1 cells infected in duplicate (1.2) with the recombinant vaccinia virus VV-TN-Ssol at an M.O.I. of 2 were harvested 18 hours after infection. The supernatant of CV1 cells infected with the virus VV-TN was also harvested as a control (M).

[0320] All the supernatants were separated on 8% SDS acrylamide gel according to Laemmli and analyzed by Western blotting with the aid of an anti-FLAG mouse monoclonal antibody and an anti-mouse IgG(H+L) polyclonal antibody coupled to peroxidase (NA931V, Amersham) (A) or with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham) (B).

[0321] A molecular mass ladder (kDa) is presented in the figure.

[0322] FIG. 37 shows the analysis of the Ssol polypeptide, purified on SDS polyacrylamide gel

[0323] 10, 5 and 211 of recombinant Ssol polypeptide purified by anti-FLAG affinity chromatography were separated on 4 to 15% gradient SDS polyacrylamide gel. The Ssol polypeptide and variable quantities of molecular mass markers (MM) were visualized by staining with silver nitrate (Gelcode SilverSNAP stain kit II, Pierce).

[0324] FIG. 38 illustrates the immunoreactivity of the recombinant Ssol polypeptide produced by the recombinant vaccinia virus VV-TN-Ssol toward sera of patients suffering from SARS. The reactivity of sera from patients was analyzed by indirect ELISA test against solid phases prepared with the aid of the purified recombinant Ssol polypeptide. The antibodies from patients reacting with the solid phase at a dilution of 1/100 and 1/400 are visualized with a human anti-IgG(H+L) polyclonal antibody coupled to peroxidase (Amersham NA933V) and TMB plus H202 (KPL). The sera of probable SARS cases are identified by a National Reference Center for Influenza Virus serial number and by the initials of the patient and the number of days elapsed since the onset of symptoms, where appropriate. The TV sera are control sera from subjects which were collected in France before the SARS epidemic which occurred in 2003.

[0325] FIG. 39 shows the anti-SARS-CoV antibody response in mice after immunization with the recombinant vaccinia viruses. Groups of 7 BALB/c mice were immunized by the i.v. route twice at 4 weeks' interval with 106 pfu of recombinant vaccinia viruses VV-TG, VV-TG-HA, VV-TG-S, VV-TG-Ssol, W-TN, W-TN-S, VV-TN-Ssol.

[0326] A. Pools of immune sera collected 3 weeks after each of the two immunizations were prepared for each of the groups and were analyzed by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG polyclonal antibody coupled to peroxidase (NA931V, Amersham) and TMB (KPL).

[0327] B. The pools of immune sera were evaluated for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

[0328] FIG. 40 describes the construction of the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol.

[0329] A. The measles vector is a complete genome of the Schwarz vaccine strain of the measles virus (MV) into which an additional transcription unit has been introduced (Combredet, 2003, Journal of Virology, 77: 11546-11554). The expression of the additional open reading frames (ORF) is controlled by cis-acting elements necessary for the transcription, for the formation of the cap and for the polyadenylation of the transgene which were copied from the elements present at the N/P junction. 2 different vectors allow the insertion between the P (phosphoprotein) and M (matrix) genes on the one hand and the H (hemagglutinin) and L (polymerase) genes on the other hand.

[0330] B. The recombinant genomes MVSchw2-SARS-S and MVSchw2-SARS-Ssol of the measles virus were constructed by inserting the ORFs of the S protein and of the Ssol polypeptide into an additional transcription unit located between the P and M genes of the vector.

[0331] The various genes of the measles virus (MV) are indicated: N (nucleoprotein), PVC (V/C phosphoprotein and protein), M (matrix), F (fusion), H (hemagglutinin), L (polymerase). T7=T7 RNA polymerase promoter, hh=hammerhead ribozyme, T7t=T7 phage RNA polymerase terminator sequence, 6=ribozyme of the hepatitis δ virus, (2), (3)= additional transcription units (ATU).

[0332] Size of the MV genome: 15 894 nt.

[0333] SP: signal peptide

[0334] TM: transmembrane region

[0335] FLAG: FLAG tag

[0336] FIG. 41 illustrates the expression of the S protein by the recombinant measles viruses, analyzed by Western blotting.

[0337] Cytoplasmic extracts were prepared after infection of Vero cells by different passages of the viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as control. Cellular extracts in loading buffer according to Laemmli were also prepared 8 hours after infection of VeroE6 cells with SARS-CoV at a multiplicity of infection of 3. They were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG(H+L) polyclonal antibody coupled to peroxidase (NA934V, Amersham).

[0338] A molecular mass ladder (kDa) is presented in the figure.

[0339] Pn: nth passage of the virus after coculture of 293-3-46 and Vero cells

[0340] SARS-CoV: extract of VeroE6 cells infected with SARS-CoV

[0341] Mock: control extract of noninfected VeroE6 cells

[0342] FIG. 42 shows the expression of the S protein by the recombinant measles viruses, analyzed by immunofluorescence

[0343] Vero cells in monolayers on glass slides were infected with the wild-type virus MWSchw (A) or the viruses MVSchw2-SARS-S (B) and MVSchw2-SARS-Ssol (C). When the syncytia have reached 30 to 40% confluence (A., B.) or 90-100% (C), the cells were fixed, permeabilized and labeled with anti-SARS-CoV rabbit polyclonal antibodies and an anti-rabbit IgG(H+L) conjugate coupled to FITC (Jackson).

[0344] FIG. 43 illustrates the Western-blot analysis of the immunoreactivity of rabbit sera directed against the peptides E1-12, E53-76 and M2-14. The rabbit 20047 was immunized with the peptide E1-12 coupled to KLH. The rabbits 22234 and 22240 were immunized with the peptide E53-76 coupled to KLH. The rabbits 20013 and 20080 were immunized with the peptide M2-14 coupled to KLH. The immune sera were analyzed by Western blotting with the aid of extracts of cells infected with SARS-CoV (B) or with the aid of extracts of cells infected with a recombinant vaccinia virus expressing the protein E (A) or M (C) of the SARS-CoV 031589 isolate. The immunoblots were visualized with the aid of an anti-rabbit IgG(H+L) conjugate coupled to peroxidase (NA934V, Amersham).

[0345] The position of the E and M proteins is indicated by an arrow.

[0346] A molecular mass ladder (kDa) is presented in the figure.

[0347] It should be understood, however, that these examples are given solely by way of illustration of the subject of the invention, and do not constitute in any manner a limitation thereto.

EXAMPLE 1

Cloning and Sequencing of the Genome of the SARS-CoV Strain Derived from the Sample Recorded Under the Number 031589

[0348] The RNA of the SARS-CoV strain was extracted from the sample of bronchoalveolar washing recorded under the number 031589, performed on a patient at the Hanoi (Vietnam) French hospital suffering from SARS.

[0349] The isolated RNA was used as template to amplify the cDNAs corresponding to the various open reading frames of the genome (ORF1a, ORF1b, ORF-S, ORF-E, ORF-M, ORF-N (including ORF-13 and ORF-14), ORF3, ORF4, ORF7 to ORF11), and at the noncoding 5' and 3' ends. The sequences of the primers and of the probes used for the amplification/detection were defined based on the available SARS-CoV nucleotide sequence.

[0350] In the text which follows, the primers and the probes are identified by: the letter S, followed by a letter which indicates the corresponding region of the genome (L for the 5' end including ORF1a and ORF1b; S, M and N for ORF-S, ORF-M, ORF-N, SE and MN for the corresponding intergene regions), and then optionally by Fn, Rn, with n between 1 and 6 corresponding to the primers used for the nested PCR (F1+R1 pair for the first amplification, F2+R2 pair for the second amplication, and the like), and then by /+/or /−/ corresponding to a sense or antisense primer and finally by the positions of the primers with reference to the Genbank sequence AY27411.3; for the sense and antisense S and N primers and the other sense primers only, when a single position is indicated, it corresponds to that of the 5' end of a probe or of a primer of about 20 bases; for the antisense primers other than the S and N primers, when a single position is indicated, it corresponds to that of the 3' end of a probe or of a primer of about 20 bases.

[0351] The amplification products thus generated were sequenced with the aid of specific primers in order to determine the complete sequence of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589. These amplification products, with the exception of those corresponding to ORF1a and ORF1b, were then cloned into expression vectors in order to produce the corresponding viral proteins and the antibodies directed against these proteins, in particular by DNA-based immunization.

1. Extraction of the RNAs

[0352] The RNAs were extracted with the aid of the Qlamp viral RNA extraction mini kit (QIAGEN) according to the manufacturer's recommendations. More specifically: 14011 of the sample and 560 μl of AVL buffer were vigorously mixed for 15 seconds, incubated for 10 minutes at room temperature and then briefly centrifuged at maximum speed. 560 μl of 100% ethanol were added to the supernatant and the mixture thus obtained was very vigorously stirred for 15 sec. 630 μl of the mixture were then deposited on the column.

[0353] The column was placed on a 2 ml tube, centrifuged for 1 min at 8000 rpm, and then the remainder of the preceding mixture was deposited on the same column, centrifuged again, for 1 min at 8000 rpm, and the column was transferred over a clean 2 ml tube. Next, 500 μl of AW1 buffer were added to the column, and then the column was centrifuged for 1 min at 8000 rpm and the eluate was discarded. 500 μl of AW2 buffer were added to the column which was then centrifuged for 3 min at 14 000 rpm and transferred onto a 1.5 ml tube. Finally, 60 μl of AVE buffer were added to the column which was incubated for 1 to 2 min at room temperature and then centrifuged for 1 min at 8000 rpm. The eluate corresponding to the purified RNA was recovered and frozen at −20° C.

2. Amplification, Sequencing and Cloning of the cDNAs

2.1) cDNA Encoding the S Protein

[0354] The RNAs extracted from the sample were subjected to reverse transcription with the aid of random sequence hexameric oligonucleotides (pdN6), so as to produce cDNA fragments.

[0355] The sequence encoding the SARS-CoV S glycoprotein was amplified in the form of two overlapping DNA

fragments: 5' fragment (SARS-Sa, SEQ ID NO: 5) and 3' fragment (SARS-Sb, SEQ ID NO: 6), by carrying out two successive amplifications with the aid of nested primers. The amplicons thus obtained were sequenced, cloned into the PCR plasmid vector 2.1-TOPO™ (INVITROGEN), and then the sequence of the cloned cDNAs was determined.

a) Cloning and Sequencing of the Sa and Sb Fragments

a.1) Synthesis of the cDNA

[0356] The reaction mixture containing: RNA (5 μl), H_2O for injection (3.5 μl), 5× reverse transcriptase buffer (4 μl), 5 mM dNTP (2 μl), pdN6 100 μg/ml (4 μl), RNasin 40 IU/μl (0.5 μl) and reverse transcriptase AMV-RT, 10 IU/μl, PROMEGA (1 μl) was incubated in a thermocycler under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and then the cDNA obtained was kept at +4° C.

a.2) First PCR Amplification

[0357] The 5' and 3' ends of the S gene were respectively amplified with the pairs of primers S/F1/+/21350-21372 and S/R1/−/23518-23498, S/F3/+/23258-23277 and S/R3/−/25382-25363. The 50 μl reaction mixture containing: cDNA (2 μl), 50 μM primers (0.5 μl), 10× buffer (5 μl), 5 mM dNTP (2 μl), Taq Expand High Fidelity, Roche (0.75 μl) and H_2O (39, 75 μl) was amplified in a thermocycler, under the following conditions: an initial step of denaturation at 94° C. for 2 min was followed by 40 cycles comprising: a step of denaturation at 94° C. for 30 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 2 min 30 sec, with 10 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 5 min.

a.3) Second PCR Amplification

[0358] The products of the first PCR amplification (5' and 3' amplicons) were subjected to a second PCR amplification step (nested PCR) under conditions identical to those of the first amplification, with the pairs of primers S/F2/+/21406-21426 and S/R2/−/23454-23435 and S/F4/+/23322-23341 and S/R4/−/25348-25329, respectively for the 5' amplicon and the 3' amplicon.

a.4) Cloning and Sequencing of the Sa and Sb Fragments

[0359] The Sa (5' end) and Sb (3' end) amplicons thus obtained were purified with the aid of the QIAquick PCR purification kit (QIAGEN), following the manufacturer's instructions, and then they were cloned into the vector PCR2.1-TOPO (Invitrogen kit), to give the plasmids called SARS-S1 and SARS-S2.

[0360] The DNA of the Sa and Sb clones was isolated and then the corresponding insert was sequenced with the aid of the Big Dye kit, Applied Biosystem® and universal primers M13 forward and M13 reverse, and primers: S/S/+/21867, S/S/+/22353, S/S/+/22811, S/S/+/23754, S/S/+/24207, S/S/+/24699, S/S/+/24348, S/S/−/24209, S/S/−/23630, S/S/−/23038, S/S/−/22454, S/S/−/21815, S/S/−/24784, S/S/+/21556, S/S/+/23130 and S/S/+/24465 following the manufacturer's instructions; the sequences of the Sa and Sb fragments thus obtained correspond to the sequences SEQ ID NO: 5 and SEQ ID NO: 6 in the sequence listing appended as an annex.

[0361] The plasmid, called SARS-S1, was deposited under the No. I-3020, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 5' fragment of the sequence of the S gene of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment called Sa corresponding to the nucleotides at positions 21406 to 23454 (SEQ ID NO: 5), with reference to the Genbank sequence AY274119.3 Tor2.

[0362] The plasmid, called TOP10F'-SARS-S2, was deposited under the No. I-3019, on May 12, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains a 3' fragment of the sequence of the S gene of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said fragment called Sb corresponding to the nucleotides at positions 23322 to 25348 (SEQ ID NO: 6), with reference to the Genbank sequence accession No. AY274119.3.

b) Cloning and Sequencing of the Complete cDNA (SARS-S Clone of 4 kb)

[0363] The complete S cDNA was obtained from the abovementioned clones SARS-S1 and SARS-S2, in the following manner:

[0364] 1) A PCR amplification reaction was carried out on a SARS-S2 clone in the presence of the above-mentioned primer S/R4/−/25348-25329 and of the primer S/S/+/24696-24715: an amplicon of 633 bp was obtained,

[0365] 2) Another PCR amplification reaction was carried out on another SARS-S2 clone, in the presence of the primers S/F4/+/23322-23341 mentioned above and S/S/−/24803-24784: an amplicon of 1481 bp was obtained.

[0366] The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, with the exception that 30 amplification cycles comprising a step of denaturation at 94° C. for 20 sec and a step of extension at 72° C. for 2 min 30 sec were carried out.

[0367] 3) The 2 amplicons (633 bp and 1481 bp) were purified under the conditions as defined above for the Sa and Sb fragments.

[0368] 4) Another PCR amplification reaction with the aid of the abovementioned primers S/F4/+/23322-23341 and S/R4/−/25348-25329 was carried out on the purified amplicons obtained in 3). The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, except that 30 amplification cycles were performed.

[0369] The 2026 bp amplicon thus obtained was purified, cloned into the vector PCR2.1-TOPO and then sequenced as above, with the aid of the primers as defined above for the Sa and Sb fragments. The clone thus obtained was called clone 3'.

[0370] 5) The clone SARS-S1 obtained above and the clone 3' were digested with EcoR I, the bands of about 2 kb thus obtained were gel purified and then amplified by PCR with the abovementioned primers S/F2/+/21406-21426 and S/R4/−/25348-25329. The amplification reaction was carried out under the conditions as defined above for the amplification of the Sa and Sb fragments, except that 30 amplification cycles were performed. The amplicon of about

4 kb was purified and sequenced. It was then cloned into the vector PCR2.1-TOPO in order to give the plasmid, called SARS-S, and the insert obtained in this plasmid was sequenced as above, with the aid of the primers as defined above for the Sa and Sb fragments. The cDNA sequences of the insert and of the amplicon encoding the S protein correspond respectively to the sequences SEQ ID NO: 4 and SEQ ID NO: 2 in the sequence listing appended as an annex, they encode the S protein (SEQ ID NO: 3).

[0371] The sequence of the amplicon corresponding to the cDNA encoding the S protein of the SARS-CoV strain derived from the sample No. 031589 has the following two mutations compared with the corresponding sequences of respectively the Tor2 and Urbani isolates, the positions of the mutations being indicated with reference to the complete sequence of the genome of the Tor2 isolate (Genbank AY274119.3):

[0372] g/t in position 23220; the alanine codon (gct) in position 577 of the amino acid sequence of the S protein of Tor2 is replaced with a serine codon (tct),

[0373] c/t in position 24872: this mutation does not modify the amino acid sequence of the S protein, and

the plasmid, called SARS-S, was deposited under the No. I-3059, on Jun. 20, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the S protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, said sequence corresponding to the nucleotides at positions 21406 to 25348 (SEQ ID NO: 4), with reference to the Genbank sequence AY274119.3.

2.2) cDNA Encoding the M and E Proteins

[0374] The RNAs derived from the sample 031589, extracted as above, were subjected to a reverse transcription, combined, during the same step (Titan One Step RT-PCR® kit, Roche), with a PCR amplification reaction, with the aid of the pairs of primers:

[0375] S/E/F1/+/26051-26070 and S/E/R1/−/26455-26436 in order to amplify ORF-E, and

[0376] S/M/F1/+/26225-26244 and S/M/R1/−/27148-27129 in order to amplify ORF-M.

[0377] A first reaction mixture containing: 8.6 μl of H_2O for injection, 1 μl of dNTP (5 mM), 0.2 μl of each of the primers (50 μM), 1.25 μl of DTT (100 mM) and 0.25 μl of RNAsin (40 IU/μl) was combined with a second reaction mixture containing: 1 μl of RNA, 7 μl of H_2O for injection, 5 μl of 5×RT-PCR buffer and 0.5 μl of enzyme mixture and the combined mixtures were incubated in a thermocycler under the following conditions: 30 min at 42° C., 10 min at 55° C., 2 min at 94° C. followed by 40 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 55° C. for 30 sec and a step of extension at 68° C. for 45 sec, with 3 sec increment per cycle and finally a step of terminal extension at 68° C. for 7 min.

[0378] The amplification products thus obtained (M and E amplicons) were subjected to a second PCR amplification (nested PCR) using the Expand High-Fi® kit, Roche), with the aid of the pairs of primers:

[0379] S/E/F2/+/26082-26101 and S/E/R2/−/26413-26394 for the amplicon E, and

[0380] S/M/F2/+/26330-26350 and S/M/R2/−/27098-27078 for the amplicon M.

[0381] The reaction mixture containing: 2 μl of the product of the first PCR, 39.25 μl of H_2O for injection, 5 μl of 10× buffer containing $MgCl_2$, 2 μl of dNTP (5 mM), 0.5 μl of each of the primers (50 μM) and 0.75 μl of enzyme mixture was incubated in a thermocycler under the following conditions: a step of denaturation at 94° C. for 2 min was followed by 30 cycles comprising a step of denaturation at 94° C. for 15 sec, a step of annealing at 60° C. for 30 sec and a step of extension at 72° C. for 45 sec, with 3 sec increment per cycle, and finally a step of terminal extension at 72° C. for 7 min. The amplification products obtained corresponding to the cDNAs encoding the E and M proteins were sequenced as above, with the aid of the primers: S/E/F2/+/26082 and S/E/R2/−/26394, S/M/F2/+/26330, S/M/R2/−/27078 cited above and the primers S/M/+/26636-26655 and S/M/−/26567-26548. They were then cloned, as above, in order to give the plasmids called SARS-E and SARS-M. The DNA of these clones was then isolated and sequenced with the aid of the universal primers M13 forward and M13 reverse and the primers S/M/+/26636 and S/M/−/26548 mentioned above.

[0382] The sequence of the amplicon representing the cDNA encoding the E protein (SEQ ID NO: 13) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani. The sequence of the E protein of the SARS-CoV 031589 strain corresponds to the sequence SEQ ID NO: 14 in the sequence listing appended as an annex.

[0383] The plasmid, called SARS-E, was deposited under the No. I-3046, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence encoding the E protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 26082 to 26413 (SEQ ID NO: 15), with reference to the Genbank sequence accession No. AY274119.3.

[0384] The sequence of the amplicon representing the cDNA encoding M (SEQ ID NO: 16) from the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequence of the isolate AY274119.3-Tor2. By contrast, at position 26857, the isolate AY278741-Urbani contains a c and the sequence of the SARS-CoV strain derived from the sample recorded under the No. 031589 contains a t. This mutation results in a modification of the amino acid sequence of the corresponding protein: at position 154, a proline (AY278741-Urbani) is changed to serine in the SARS-CoV strain derived from the sample recorded under the No. 031589. The sequence of the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 17 in the sequence listing appended as an annex.

[0385] The plasmid, called SARS-M, was deposited under the No. I-3047, on May 28, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux,

75724 Paris Cedex 15; it contains the cDNA sequence encoding the M protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above; said sequence corresponding to the nucleotides at positions 26330 to 27098 (SEQ ID NO: 18), with reference to the Genbank sequence accession No. AY274119.3.

2.3) cDNA Corresponding to ORF3, ORF4, ORF7 to ORF11

[0386] The same amplification, cloning and sequencing strategy was used to obtain the cDNA fragments corresponding respectively to the following ORFs: ORF3, ORF4, ORF7, ORF8, ORF9, ORF10 and ORF11. The pairs of primers used for the first amplification are:

[0387] ORF3 and ORF4: S/SE/F1/+/25069-25088 and S/SE/R1/−/26300-26281

[0388] ORF7 to ORF11: S/MN/F1/+/26898-26917 and S/MN/R1/−/28287-28266

[0389] The pairs of primers used for the second amplification are:

[0390] ORF3 and ORF4: S/SE/F2/+/25110-25129 and S/SE/R2/−/26244-26225

[0391] ORF7 to ORF11: S/MN/F2/+/26977-26996 and S/MN/R2/−/28218-28199

[0392] The conditions for the first amplification (RT-PCR) are the following: 45 min at 42° C., 10 min at 55° C., 2 min at 94° C. followed by 40 cycles comprising a step of denaturation at 94° C. for 15 sec, a step of annealing at 58° C. for 30 sec and a step of extension at 68° C. for 1 min, with 5 sec increment per cycle and finally a step of terminal extension at 68° C. for 7 min.

[0393] The conditions for the nested PCR are the following: a step of denaturation at 94° C. for 2 min was followed by 40 cycles comprising a step of denaturation at 94° C. for 20 sec. a step of annealing at 58° C. for 30 sec and a step of extension at 72° C. for 50 sec, with 4 sec increment per cycle and finally a step of terminal extension at 72° C. for 7 min.

[0394] The amplification products obtained corresponding to the cDNAs containing respectively ORF3 and 4 and ORF7 to 11 were sequenced with the aid of the primers: S/SE/+/25363, S/SE/+/25835, S/SE/−/25494, S/SE/−/25875, S/MN/+/27839, S/MN/+/27409, S/MN/−/27836, S/MN/−/27799 and cloned as above for the other ORFs, to give the plasmids called SARS-SE and SARS-MN. The DNA of these clones was isolated and sequenced with the aid of these same primers and of the universal primers M13 sense and M13 antisense.

[0395] The sequence of the amplicon representing the cDNA of the region containing OFR3 and ORF4 (SEQ ID NO: 7) of the SARS-CoV strain derived from the sample No. 031589 contains a nucleotide difference in relation to the corresponding sequence of the isolate AY274119-Tor2. This mutation at position 25298 results in a modification of the amino acid sequence of the corresponding protein (ORF3): at position 11, an arginine (AY274119-Tor2) is changed to glycine in the SARS-CoV strain derived from the sample No. 031589. By contrast, no mutation was identified in relation to the corresponding sequence of the isolate AY278741-Urbani. The sequences of ORF3 and 4 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 10 and 12 in the sequence listing appended as an annex.

[0396] The plasmid, called SARS-SE, was deposited under the No. I-3126, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the region situated between ORF-S and ORF-E and overlapping ORF-E of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said region corresponding to the nucleotides at positions 25110 to 26244 (SEQ ID NO: 8), with reference to the Genbank sequence accession No. AY274119.3.

[0397] The sequence of the amplicon representing the cDNA corresponding to the region containing ORF7 to ORF11 (SEQ ID NO: 19) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119-Tor2 and AY278741-Urbani. The sequences of ORF7 to 11 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 22, 24, 26, 28 and 30 in the sequence listing appended as an annex.

[0398] The plasmid, called SARS-MN, was deposited under the No. I-3125, on Nov. 13, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the region situated between ORF-M and ORF-N of the SARS-CoV strain derived from the sample recorded under the No. 031589 and collected in Hanoi, as defined above, said sequence corresponding to the nucleotides at positions 26977 to 28218 (SEQ ID NO: 20), with reference to the Genbank sequence accession No. AY274119.3.

[0399] The sequence of the amplicon representing the cDNA corresponding to the region containing ORF7 to ORF11 (SEQ ID NO: 19) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119-Tor2 and AY278741-Urbani. The sequences of ORF7 to 11 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 22, 24, 26, 28 and 30 in the sequence listing appended as an annex.

2.4) cDNA Encoding the N Protein and Including ORF13 and ORF14

[0400] The cDNA was synthesized and amplified as described above for the fragments Sa and Sb. More specifically, the reaction mixture containing: 5 μl of RNA, 5 μl of H_2O for injection, 4 μl of 5× reverse transcriptase buffer, 2 μl of dNTP (5 mM), 2 μl of oligo 20T (5 μM), 0.5 μl of RNasin (40 IU/μl) and 1.5 μl of AMV-RT (10 IU/μl Promega) was incubated in a thermocycler under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and it was then kept at +4° C.

[0401] A first PCR amplification was performed with the pair of primers S/N/F3/+/28023 and S/N/R3/−/29480.

[0402] The reaction mixture as above for the amplification of the S1 and S2 fragments was incubated in a thermocycler, under the following conditions: an initial step of denaturation at 94° C. for 2 min was followed by 40 cycles

comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 1 min 30 sec with 10 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 5 min.

[0403] The amplicon obtained at the first PCR amplification was subjected to a second PCR amplification step (nested PCR) with the pairs of primer S/N/F4/+/28054 and S/N/R4/−/29430 under conditions identical to those of the first amplification.

[0404] The amplification product obtained, corresponding to the cDNA encoding the N protein of the SARS-CoV strain derived from the sample No. 031589, was sequenced with the aid of the primers: S/N/F4/+/28054, S/N/R4/−/29430, S/N/+/28468, S/N/+/28918 and S/N/−/28607 and cloned as above for the other ORFs, to give the plasmid called SARS-N. The DNA of these clones was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense, and the primers S/N/+/28468, S/N/+/28918 and S/N/−/28607.

[0405] The sequence of the amplicon representing the cDNA corresponding to ORF-N and including ORF13 and ORF14 (SEQ ID NO: 36) of the SARS-CoV strain derived from the sample No. 031589 does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani. The sequence of the N protein of the SARS-CoV strain derived from the sample No. 031589 corresponds to the sequence SEQ ID NO: 37 in the sequence listing appended as an annex.

[0406] The sequences of ORF13 and 14 of the SARS-CoV strain derived from the sample No. 031589 correspond respectively to the sequences SEQ ID NO: 32 and 34 in the sequence listing appended as an annex.

[0407] The plasmid, called SARS-N, was deposited under the No. I-3048, on Jun. 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA encoding the N protein of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 28054 to 29430 (SEQ ID NO: 38), with reference to the Genbank sequence accession No. AY274119.3.

2.5) Noncoding 5' and 3' Ends

a) Noncoding 5' end (5'NC)

a_1) Synthesis of the cDNA

[0408] The RNAs derived from the sample 031589, extracted as above, were subjected to reverse transcription under the following conditions:

[0409] The RNA (15 μl) and the primer S/L/−/443 (3 μl at the concentration of 5 μm) were incubated for 10 min at 75° C.

[0410] Next, the 5× reverse transcriptase buffer (6 μl, INVITROGEN), 10 Mm dNTP (1 μl), 0.1 M DTT (3 μl) were added and the mixture was incubated at 50° C. for 3 min.

[0411] Finally, the reverse transcriptase (3 μl of Superscript®, INVITROGEN) was added to the preceding mixture which was incubated at 50° C. for 1 h 30 min and then at 90° C. for 2 min.

[0412] The cDNA thus obtained was purified with the aid of the QIAquick PCR purification kit (QIAGEN), according to the manufacturer's recommendations.

b_1) Terminal Transferase Reaction (TdT)

[0413] The cDNA (10 μl) is incubated for 2 min at 100° C., stored in ice, and the following are then added: H_2O (2.5 μl), 5×TdT buffer (4 μl, AMERSHAM), 5 mM dATP (2 μl) and TdT (1.5 μl, AMERSHAM). The mixture thus obtained is incubated for 45 min at 37° C. and then for 2 min at 65° C.

[0414] The product obtained is amplified by a first PCR reaction with the aid of the primers: S/L/−225-206 and anchor 14T: 5'-AGATGAATTCGGTAC-CTTTTTTTTTTTTTT-3' (SEQ ID NO: 68). The amplification conditions are the following: an initial step of denaturation at 94° C. for 2 min is followed by 10 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 45° C. for 30 sec and then a step of extension at 72° C. for 30 sec and then by 30 cycles comprising a step of denaturation at 94° C. for 10 sec, a step of annealing at 50° C. for 30 sec and then a step of extension at 72° C. for 30 sec, and then a final step of extension at 72° C. for 5 min.

[0415] The product of the first PCR amplification was subjected to a second amplification step with the aid of the primers: S/L/−/204-185 and anchor 14T mentioned above under conditions identical to those of the first amplification. The amplicon thus obtained was purified, sequenced with the aid of the primer S/L/−/182-163 and it was then cloned as above for the different ORFs, to give the plasmid called SARS-5'NC. The DNA of this clone was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense and the primer S/L/−/182-163 mentioned above.

[0416] The amplicon representing the cDNA corresponding to the 5'NC end of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 72 in the sequence listing appended as an annex; this sequence does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani.

[0417] The plasmid, called SARS-5'NC, was deposited under the No. I-3124, on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA corresponding to the noncoding 5' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to the nucleotides at positions 1 to 204 (SEQ ID NO: 39), with reference to the Genbank sequence accession No. AY274119.3.

b) Noncoding 3' End (3'NC)

a_1) Synthesis of the cDNA

[0418] The RNAs derived from the sample 031589, extracted as above, were subjected to reverse transcription, according to the following protocol: the reaction mixture containing: RNA (5 μl), H_2O (5 μl), 5× reverse transcriptase buffer (4 μl), 5 mM dNTP (2 μl), 5 μM Oligo 20T (2 μl), 40 U/μl RNasin (0.5 μl) and 10 IU/μl RT-AMV (1.5 μl, PROMEGA) was incubated in a thermo-cycler, under the following conditions: 45 min at 42° C., 15 min at 55° C., 5 min at 95° C., and it was then kept at +4° C.

[0419] The cDNA obtained was amplified by a first PCR reaction with the aid of the primers S/N/+/28468-28487 and anchor 14T mentioned above. The amplification conditions are the following: an initial step of denaturation at 94° C. for 2 min is followed by 10 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 45° C. for 30 sec and then a step of extension at 72° C. for 50 sec and then 30 cycles comprising a step of denaturation at 94° C. for 20 sec, a step of annealing at 50° C. for 30 sec and then a step of extension at 72° C. for 50 sec, and then a final step of extension at 72° C. for 5 min.

[0420] The product of the first PCR amplification was subjected to a second amplification step with the aid of the primers S/N/+/28933-28952 and anchor 14T mentioned above, under conditions identical to those of the first amplification. The amplicon thus obtained was purified, sequenced with the aid of the primer S/N/+/29257-29278 and cloned as above for the different ORFs, to give the plasmid called SARS-3'NC. The DNA of this clone was isolated and sequenced with the aid of the universal primers M13 sense and M13 antisense and the primer S/N/+/29257-29278 mentioned above.

[0421] The amplicon representing the cDNA corresponding to the 3'NC end of the SARS-CoV strain derived from the sample recorded under the No. 031589 corresponds to the sequence SEQ ID NO: 73 in the sequence listing appended as an annex; this sequence does not contain differences in relation to the corresponding sequences of the isolates AY274119.3-Tor2 and AY278741-Urbani.

[0422] The plasmid called SARS-3'NC was deposited under the No. I-3123 on Nov. 7, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15; it contains the cDNA sequence corresponding to the noncoding 3' end of the genome of the SARS-CoV strain derived from the sample recorded under the No. 031589, as defined above, said sequence corresponding to that situated between the nucleotide at positions 28933 to 29727 (SEQ ID NO: 40), with reference to the Genbank sequence accession No. AY274119.3, ends with a series of nucleotides a.

2.6) ORF1a and ORF1b

[0423] The amplification of the 5' region containing ORF1a and ORF1b of the SARS-CoV genome derived from the sample 031589 was performed by carrying out RT-PCR reactions followed by nested PCRs according to the same principles as those described above for the other ORFs. The amplified fragments overlap over several tenths of bases, thus allowing computer reconstruction of the complete sequence of this part of the genome. On average, the amplified fragments are of two kilobases.

[0424] 14 overlapping fragments, called L0 to L12, were thus amplified with the aid of the following primers:

TABLE II

Primers used for the amplification of the 5' region (ORF1a and ORF1b)

REGION AMPLIFIED AND SEQUENCED (does not include the primers)	RT-PCR sense primer	RT-PCR antisense primer	Nested PCR sense primer	Nested PCR antisense primer
L0 50-480	S/L0/F1/+30	S/L0/R1/−481		
L1 231-2240	S/L1/F1/+147	S/L1/R1/−2338	S/L1/F2/+211	S/L1/R2/−2241
L2 2156-4167	S/L2/F1/+2033	S/L2/R1/−4192	S/L2/F2/+2136	S/L2/R2/−4168
L3 3913-5324	S/L3bis/F1/+3850	S/L3bis/R1/−5365	S/L3bis/F2/+3892	S/L3bis/R2/−5325
L4b 4952-6023	S/L4b/F1/+4878	S/L4b/R1/−6061	S/L4b/F2/+4932	S/L4b/R2/−6024
L4 5325-7318	S/L4/F1/+5272	S/L4/R1/−7392	S/L4/F2/+5305	S/L4/R2/−7323
L5 7296-9156	S/L5/F1/+7111	S/L5/R1/−9253	S/L5/F2/+7275	S/L5/R2/−9157
L6 9053-11066	S/L6/F1/+8975	S/L6/R1/−11151	S/L6/F2/+9032	S/L6/R2/−11067
L7 10928-12962	S/L7/F1/+10883	S/L7/R1/−13050	S/L7/F2/+10928	S/L7/R2/−12963
L8 12835-14834	S/L8/F1/+12690	S/L8/R1/−14857	S/L8/F2/+12815	S/L8/R2/−14835
L9 14765-16624	S/L9/F1/+14688	S/L9/R1/−16678	S/L9/F2/+14745	S/L9/R2/−16625
L10 16534-18570	S/L10/F1/+16451	S/L10/R1/−18594	S/L10/F2/+16514	S/L10/R2/−18571
L11 18521-20582	S/L11/F1/+18441	S/L11/R1/−20612	S/L11/F2/+18500	S/L11/R2/−20583
L12 20338-22205.	S/L12/F1/+20279	S/L12/R1/−22229	S/L12/F2/+20319	S/L12/R2/−22206

[0425] All the fragments were amplified under the following conditions, except fragment L0 which was amplified as described above for ORF-M:

[0426] RT-PCR: 30 min at 42° C., 15 min at 55° C., 2 min at 94° C., and then the cDNA obtained is amplified under the following conditions: 40 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 58° C. for 30 sec and then a step of extension at 68° C. for 1 min 30 sec, with 5 sec additional extension at each cycle, and then a final step of extension at 68° C. for 7 min.

[0427] Nested PCR: An initial step of denaturation at 94° C. for 2 min is followed by 35 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 60° C. for 30 sec and then a step of extension at 72° C. for 1 min 30 sec, with 5 sec of additional extension at each cycle, and then a final step of extension at 72° C. for 7 min.

[0428] The amplification products were sequenced with the aid of the primers defined in table III below:

TABLE III

Primers used for the sequencing of the 5' region (ORF1a and ORF1b)

Names	Sequences (SEQ ID NO: 76 to 139)
S/L3/+/4932	5'-CCACACACAGCTTGTGGATA-3'
S/L4/+/6401	5'-CCGAAGTTGTAGGCAATGTC-3'
S/L4/+/6964	5'-TTTGGTGCTCCTTCTTATTG-3'
S/L4/-/6817	5'-CCGGCATCCAAACATAATTT-3'
S/L5/-/7633	5'-TGGTCAGTAGGGTTGATTGG-3'
S/L5/-/8127	5'-CATCCTTTGTGTCAACATCG-3'
S/L5/-/8633	5'-GTCACGAGTGACACCATCCT-3'
S/L5/+/7839	5'-ATGCGACGAGTCTGCTTCTA-3'
S/L5/+/8785	5'-TTCATAGTGCCTGGCTTACC-3'
S/L5/+/8255	5'-ATCTTGGCGCATGTATTGAC-3'
S/L6/-/9422	5'-TGCATTAGCAGCAACAACAT-3'
S/L6/-/9966	5'-TCTGCAGAACAGCAGAAGTG-3'
S/L6/-/10542	5'-CCTGTGCAGTTTGTCTGTCA-3'
S/L6/+/10677	5'-CCTTGTGGCAATGAAGTACA-3'
S/L6/+/10106	5'-ATGTCATTTGCACAGCAGAA-3'
S/L6/+/9571	5'-CTTCAATGGTTTGCCATGTT-3'
S/L7/-/11271	5'-TGCGAGCTGTCATGAGAATA-3'
S/L7/-/11801	5'-AACCGAGAGCAGTACCACAG-3'
S/L7/-/12383	5'-TTTGGCTGCTGTAGTCAATG-3'
S/L7/+/12640	5'-CTACGACAGATGTCCTGTGC-3'
S/L7/+/12088	5'-GAGCAGGCTGTAGCTAATGG-3'
S/L7/+/11551	5'-TTAGGCTATTGTTGCTGCTG-3'

TABLE III-continued

Primers used for the sequencing of the 5' region (ORF1a and ORF1b)

Names	Sequences (SEQ ID NO: 76 to 139)
S/L8/-13160	5'-CAGACAACATGAAGCACCAC-3'
S/L8/-/13704	5'-CGCTGACGTGATATATGTGG-3'
S/L8/-14284	5'-TGCACAATGAAGGATACACC-3'
S/L8/+/14453	5'-ACATAGCTCGCGTCTCAGTT-3'
S/L8/+/13968	5'-GGCATTGTAGGCGTACTGAC-3'
S/L8/+/13401	5'-GTTTGCGGTGTAAGTGCAG-3'
S/L9/-15098	5'-TAGTGGCGGCTATTGACTTC-3'
S/L9/-15677	5'-CTAAACCTTGAGCCGCATAG-3'
S/L9/-16247	5'-CATGGTCATAGCAGCACTTG-3'
S/L9/+16323	5'-CCAGGTTGTGATGTCACTGAT-3'
S/L9/+15858	5'-CCTTACCCAGATCCATCAAG-3'
S/L9/+15288	5'-CGCAAACATAACACTTGCTG-3'
S/L10/-16914	5'-AGTGTTGGGTACAAGCCAGT-3'
S/L10/-17466	5'-GTTCCAAGGAACATGTCTGG-3'
S/L10/-18022	5'-AGGTGCCTGTGTAGGATGAA-3'
S/L10/+18245	5'-GGGCTGTCATGCAACTAGAG-3'
S/L10/+17663	5'-TCTTACACGCAATCCTGCTT-3'
S/L10/+17061	5'-TACCCATCTGCTCGCATAGT-3'
S/L11/-/18877	5'-GCAAGCAGAATTAACCCTCA-3'
S/L11/-19396	5'-AGCACCACCTAAATTGCATC-3'
S/L11/-20002	5'-TGGTCCCTTTGAAGGTGTTA-3'
S/L11/+20245	5'-TCGAACACATCGTTTATGGA-3'
S/L11/+/19611	5'-GAAGCACCTGTTTCCATCAT-3'
S/L11/+/19021	5'-ACGATGCTCAGCCATGTAGT-3'
SARS/L1/F3/+800	5'-GAGGTGCAGTCACTCGCTAT-3'
SARS/L1/F4/+1391	5'-CAGAGATTGGACCTGAGCAT-3'
SARS/L1/F5/+1925	5'-CAGCAAACCACTCAATTCCT-3'
SARS/L1/R3/-1674	5'-AAATGATGGCAACCTCTTCA-3'
SARS/L1/R4/-1107	5'-CACGTGGTTGAATGACTTTG-3'
SARS/L1/R5/-520	5'-ATTTCTGCAACCAGCTCAAC-3'
SARS/L2/F3/+2664	5'-CGCATTGTCTCCTGGTTTAC-3'
SARS/L2/F4/+3232	5'-GAGATTGAGCCAGAACCAGA-3'
SARS/L2/F5/+3746	5'-ATGAGCAGGTTGTCATGGAT-3'
SARS/L2/R3/-3579	5'-CTGCCTTAAGAAGCTGGATG-3'
SARS/L2/R4/-2991	5'-TTTCTTCACCAGCATCATCA-3'

TABLE III-continued

Primers used for the sequencing of the 5' region (ORF1a and ORF1b)

Names	Sequences (SEQ ID NO: 76 to 139)
SARS/L2/R5/-2529	5'-CACCGTTCTTGAGAACAACC-3'
SARS/L3/F3/+4708	5'-TCTTTGGCTGGCTCTTACAG-3'
SARS/L3/F4/+5305	5'-GCTGGTGATGCTGCTAACTT-3'
SARS/L3/F5/+5822	5'-CCATCAAGCCTGTGTCGTAT-3'
SARS/L3/R3/-5610	5'-CAGGTGGTGCAGACATCATA-3'
SARS/L3/R4/-4988	5'-AACATCAGCACCATCCAAGT-3'
SARS/L3/R5/-4437	5'-ATCGGACACCATAGTCAACG-3'

[0429] The sequences of the fragments L0 to L12 of the SARS-CoV strain derived from the sample recorded under the No. 031589 correspond respectively to the sequences SEQ ID NO: 41 to SEQ ID NO: 54 in the sequence listing appended as an annex. Among these sequences, only that corresponding to the fragments L5 contains a nucleotide difference in relation to the corresponding sequence of the isolate AY278741-Urbani. This t/c mutation at position 7919 results in a modification of the amino acid sequence of the corresponding protein, encoded by ORF1a: at position 2552, a valine (gtt codon; AY278741) is changed to alanine (gct codon) in the SARS-CoV strain 031589. By contrast, no mutation was identified in relation to the corresponding sequence of the isolate AY274119.3-Urbani. The other fragments do not exhibit differences in relation to the corresponding sequences of the isolates Tor2 and Urbani.

EXAMPLE 2

Production and Purification of the Recombinant N and S Proteins of the SARS-CoV Strain Derived from the Sample Recorded Under the Number 031589

[0430] The entire N protein and two polypeptide fragments of the S protein of the SARS-CoV strain derived from the sample recorded under the number 031589 were produced in *E. coli*, in the form of fusion proteins comprising an N- or C-terminal polyhistidine tag. In the two S polypeptides, the N- and C-terminal hydrophobic sequences of the S protein (signal peptide: positions 1 to 13 and transmembrane helix: positions 1196 to 1218) were deleted whereas the β helix (positions 565 to 687) and the two motifs of the coiled-coil type (positions 895 to 980 and 1155 to 1186) of the S protein were preserved. These two polypeptides consist of: a long fragment (S_L) corresponding to positions 14 to 1193 of the amino acid sequence of the S protein and a short fragment (S_C) corresponding to positions 475 to 1193 of the amino acid sequence of the S protein.

1) Cloning of the cDNAS N, S_L and S_C into the Expression Vectors pIVEX2.3 and pIVEX2.4

[0431] The cDNAs corresponding to the N protein and to the S_L and S_C fragments were amplified by PCR under standard conditions, with the aid of the DNA polymerase Platinum Pfx® (INVITROGEN). The plasmids SRAS-N and SRAS-S were used as template and the following oligo-nucleotides as primers:

5'-CCCATATGTCTGATAATGGACCCCAATCAAAC-3'
(N sense, SEQ ID NO: 55)

5'-CCCCCGGGTGCCTGAGTTGAATCAGCAGAAGC-3'
(N antisense, SEQ ID NO: 56)

5'-CCCATATGAGTGACCTTGACCGGTGCACCAC-3'
(S_C sense, SEQ ID NO: 57)

5'-CCCATATGAAACCTTGCACCCCACCTGCTC-3'
(S_L sense, SEQ ID NO: 58)

5'-CCCCCGGGTTTAATATATTGCTCATATTTTCCC-3'
(S_C and S_L antisense, SEQ ID NO: 29).

[0432] The sense primers introduce an NdeI site (underlined) while the antisense primers introduce an XmaI or SmaI site (underlined). The 3 amplification products were column purified (*QIAquick PCR Purification* kit, QIAGEN) and cloned into an appropriate vector. The plasmid DNA purified from the 3 constructs (*QIAFilter Midi Plasmid* kit, QIAGEN) was verified by sequencing and digested with the enzymes NdeI and XmaI. The 3 fragments corresponding to the cDNAs N, S_L and S_C were purified on agarose gel and then inserted into the plasmids pIVEX2.3MCS(C-terminal polyhistidine tag) and pIVEX2.4d (N-terminal polyhistidine tag) digested beforehand with the same enzymes. After verification of the constructs, the 6 expression vectors thus obtained (pIV2.3N, pIV2.3S_C, pIV2.3S_L, pIV2.4N, pIV2.4S_c also called pIV2.4S_1, pIV2.4S_L) were then used, on the one hand to test the expression of the proteins in vitro, and on the other hand to transform the bacterial strain BL21(DE3)pDIA17 (NOVAGEN). These constructs encode proteins whose expected molecular mass is the following: pIV2.3N (47174 Da), pIV2.3S_C (82897 Da), pIV2.3S_L (132056 Da), pIV2.4N (48996 Da), pIV2.4S_1 (81076 Da) and pIV2.4S_L (133877 Da). Bacteria transformed with pIV2.3N were deposited at the CNCM on Oct. 23, 2003, under the number I-3117, and bacteria transformed with pIV2.4S_1 were deposited at the CNCM on Oct. 23, 2003, under the number I-3118.

2) Analysis of the Expression of the Recombinant Proteins In Vitro and In Vivo

[0433] The expression of recombinant proteins from the 6 recombinant vectors was tested, in a first instance, in a system in vitro (RTS100, Roche). The proteins produced in vitro, after incubation of the recombinant vectors pIVEX for 4 h at 30° C., in the RTS100 system, were analyzed by Western blotting with the aid of an anti-$(his)_6$ antibody coupled to peroxidase. The result of expression in vitro (FIG. 1) shows that only the N protein is expressed in large quantities, regardless of the position, N- or C-terminal, of the polyhistidine tag. In a second step, the expression of the N and S proteins was tested in vivo at 30° C. in LB medium in the presence or in the absence of inducer (1 mM IPTG). The N protein is very well produced in this bacterial The sequences of the fragments L0 to L12 of the SARS-CoV strain derived from the sample recorded under the No. 031589 correspond respectively to the sequences SEQ ID NO: 41 to SEQ ID NO: 54 in the sequence listing appended

as an annex. Among these sequences, only that corresponding to the fragments L5 contains a nucleotide difference in relation to the corresponding sequence of the isolate AY278741-Urbani. This t/c mutation at position 7919 results in a modification of the amino acid sequence of the corresponding protein, encoded by ORF1a: at position 2552, a valine (gtt codon; AY278741) is changed to alanine (gct codon) in the SARS-CoV strain 031589. By contrast, no mutation was identified in relation to the corresponding sequence of the isolate AY274119.3-Urbani. The other fragments do not exhibit differences in relation to the corresponding sequences of the isolates Tor2 and Urbani.

EXAMPLE 2

Production and Purification of the Recombinant N and S Proteins of the SARS-CoV Strain Derived from the Sample Recorded Under the Number 031589

[0434] The entire N protein and two polypeptide fragments of the S protein of the SARS-CoV strain derived from the sample recorded under the number 031589 were produced in *E. coli*, in the form of fusion proteins comprising an N- or C-terminal polyhistidine tag. In the two S polypeptides, the N- and C-terminal hydrophobic sequences of the S protein (signal peptide: positions 1 to 13 and transmembrane helix: positions 1196 to 1218) were deleted whereas the β helix (positions 565 to 687) and the two motifs of the coiled-coil type (positions 895 to 980 and 1155 to 1186) of the S protein were preserved. These two polypeptides consist of: a long fragment (S_L) corresponding to positions 14 to 1193 of the amino acid sequence of the S protein and a short fragment (S_C) corresponding to positions 475 to 1193 of the amino acid sequence of the S protein.

1) Cloning of the cDNAS N, S_L and S_C into the Expression Vectors pIVEX2.3 and pIVEX2.4

[0435] The cDNAs corresponding to the N protein and to the S_L and S_C fragments were amplified by PCR under standard conditions, with the aid of the DNA polymerase Platinum Pfx® (INVITROGEN). The plasmids SRAS-N and SRAS-S were used as template and the following oligo-nucleotides as primers:

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5'-CCCATATGTCTGATAATGGACCCCAATCAAAC-3'
(N sense, SEQ ID NO: 55)

5'-CCCCCGGGTGCCTGAGTTGAATCAGCAGAAGC-3'
(N antisense, SEQ ID NO: 56)

5'-CCCATATGAGTGACCTTGACCGGTGCACCAC-3'
(Sc sense, SEQ ID NO: 57)

5'-CCCATATGAAACCTTGCACCCCACCTGCTC-3'
(SL sense, SEQ ID NO: 58)

5'-CCCCCGGGTTTAATATATTGCTCATATTTTCCC-3'
(Sc and SL antisense, SEQ ID NO: 29).
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[0436] The sense primers introduce an NdeI site (underlined) while the antisense primers introduce an XmaI or SmaI site (underlined). The 3 amplification products were column purified (*QIAquick PCR Purification* kit, QIAGEN) and cloned into an appropriate vector. The plasmid DNA purified from the 3 constructs (*QIAFilter Midi Plasmid* kit, QIAGEN) was verified by sequencing and digested with the enzymes NdeI and XmaI. The 3 fragments corresponding to the cDNAs N, S_L and S_C were purified on agarose gel and then inserted into the plasmids pIVEX2.3MCS(C-terminal polyhistidine tag) and pIVEX2.4d (N-terminal polyhistidine tag) digested beforehand with the same enzymes. After verification of the constructs, the 6 expression vectors thus obtained (pIV2.3N, pIV2.3S_C, pIV2.3S_L, pIV2.4N, pIV2.4S_C also called pIV2.4S_1, pIV2.4S_L) were then used, on the one hand to test the expression of the proteins in vitro, and on the other hand to transform the bacterial strain BL21(DE3)pDIA17 (NOVAGEN). These constructs encode proteins whose expected molecular mass is the following: pIV2.3N (47174 Da), pIV2.3S_C (82897 Da), pIV2.3S_L (132056 Da), pIV2.4N (48996 Da), pIV2.4S_1 (81076 Da) and pIV2.4S_L (133877 Da). Bacteria transformed with pIV2.3N were deposited at the CNCM on Oct. 23, 2003, under the number I-3117, and bacteria transformed with pIV2.4S_1 were deposited at the CNCM on Oct. 23, 2003, under the number I-3118.

2) Analysis of the Expression of the Recombinant Proteins In Vitro and In Vivo

[0437] The expression of recombinant proteins from the 6 recombinant vectors was tested, in a first instance, in a system in vitro (RTS100, Roche). The proteins produced in vitro, after incubation of the recombinant vectors pIVEX for 4 h at 30° C., in the RTS100 system, were analyzed by Western blotting with the aid of an anti-$(his)_6$ antibody coupled to peroxidase. The result of expression in vitro (FIG. **1**) shows that only the N protein is expressed in large quantities, regardless of the position, N- or C-terminal, of the polyhistidine tag. In a second step, the expression of the N and S proteins was tested in vivo at 30° C. in LB medium in the presence or in the absence of inducer (1 mM IPTG). The N protein is very well produced in this bacterial system (FIG. **2**) and is found mainly in a soluble fraction after lysis of the bacteria. By contrast, the long version of S (S_L) is very weakly produced and is completely insoluble (FIG. **3**). The short version (S_C) also exhibits a very weak solubility, but an expression level that is much higher than that of the long version. Moreover, the construct S_C fused with a polyhistidine tag at the C-terminal position has a smaller size than that expected. An immunodetection experiment with an anti-polyhistidine antibody has shown that this construct was incomplete. In conclusion, the two constructs, pIV2.3N and pIV2.4S_1, which express respectively the entire N protein fused with the C-terminal polyhistidine tag and the short S protein fused with the N-terminal polyhistidine tag, were selected in order to produce the two proteins in a large quantity so as to purify them. The plasmids pIV2.3N and pIV2.4S_1 were deposited respectively under the No. I-3117 and I-3118 at the CNCM, 25 rue du Docteur Roux, 75724 PARIS 15, on Oct. 23, 2003.

3) Analysis of the Antigenic Activity of the Recombinant Proteins

[0438] The antigenic activity of the N, S_L and S_C proteins was tested by Western blotting with the aid of two serum samples, obtained from the same patient infected with SARS-CoV, collected 8 days (M12) and 29 days (M13) after the onset of the SARS symptoms. The experimental protocol is as described in example 3. The results illustrated by FIG. **4** show (i) the seroconversion of the patient, and (ii) that the N protein possesses a higher antigenic reactivity than the short S protein.

4) Purification of the N Protein from pIV2.3N

[0439] Several experiments for purifying the N protein, produced from the vector pIV2.3N, were carried out according to the following protocol. The bacteria BL21(DE3)pDIA17, transformed with the expression vector pIV2.3N, were cultured at 30° C. in 1 liter of culture medium containing 0.1 mg/ml of ampicillin, and induced with 1 mM IPTG when the cell density equivalent to A_{600}=0.8 is reached (about 3 hours). After 2 hours of culture in the presence of inducer, the cells were recovered by centrifugation (10 min at 5000 rpm), resuspended in the lysis buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 20 mM imidazole, pH 8, containing the mixture of protease inhibitors Complete®, Roche), and lysed with the French press (12 000 psi). After centrifugation of the bacterial lysate (15 min at 12 000 rpm), the supernatant (50 ml) was deposited at a flow rate of 1 ml/min on a metal chelation column (15 ml) (Ni-NTA superflow, Qiagen), equilibrated with the lysis buffer. After washing the column with 200 ml of lysis buffer, the N protein was eluted with an imidazole gradient (20→250 mM) in 10 column volumes. The fractions containing the N protein were assembled and analyzed by polyacrylamide gel electrophoresis under denaturing conditions followed by staining with Coomassie blue. The results illustrated by FIG. **5** show that the protocol used makes it possible to purify the N protein with a very satisfactory homogeneity (95%) and a mean yield of 15 mg of protein per liter of culture.

5) Purification of the S_C Protein from pIV2.4S_C (pIV2.4S_1)

[0440] The protocol followed for purifying the short S protein is very different from that described above because the protein is highly aggregated in the bacterial system (inclusion bodies). The bacteria BL21(DE3)pDIA17, transformed with the expression vector pIV2.4S_1, were cultured at 30° C. in 1 liter of culture medium containing 0.1 mg/ml of ampicillin, and induced with 1 mM IPTG when the cell density equivalent to A_{600}=0.8 is reached (about 3 hours). After 2 hours of culture in the presence of inducer, the cells were recovered by centrifugation (10 min at 5000 rpm), resuspended in the lysis buffer (0.1 M Tris-HCl, 1 mM EDTA, pH 7.5), and lysed with the French press (1200 psi). After centrifugation of the bacterial lysate (15 min at 12 000 rpm), the pellet was resuspended in 25 ml of lysis buffer containing 2% Triton X100 and 10 mM β-mercaptoethanol, and then centrifuged for 20 min at 12 000 rpm. The pellet was resuspended in 10 mM Tris-HCl buffer containing 7 M urea, and gently stirred for 30 min at room temperature. This final washing of the inclusion bodies with 7 M urea is necessary in order to remove most of the *E. coli* membrane proteins which co-sediment with the aggregated S_C protein. After a final centrifugation for 20 min at 12 000 rpm, the final pellet is resuspended in the 10 mM Tris-HCl buffer. The electrophoretic analysis of this preparation (FIG. **6**) shows that the short S protein may be purified with a satisfactory homogeneity (about 90%) from the inclusion bodies (insoluble extract).

EXAMPLE 3

Immunodominance of the N Protein

[0441] The reactivity of the antibodies present in the serum of patients suffering from atypical pneumopathy caused by the SARS-associated coronavirus (SARS-CoV), toward the various proteins of this virus, was analyzed by Western blotting under the conditions described below.

1) Materials

a) Lysate of Cells Infected with SARS-CoV

[0442] Vero E6 cells (2×10^6) were infected with SARS-CoV (isolate recorded under the number FFM/MA104) at a multiplicity of infection (M.O.I.) of 10^{-1} or 10^{-2} and then incubated in DMEM medium containing 2% FCS, at 35° C. in an atmosphere containing 5% CO_2. 48 hours later, the cellular lawn was washed with PBS and then lysed with 500 μl of loading buffer prepared according to Laemmli and containing β-mercaptoethanol. The samples were then boiled for 10 minutes and then sonicated for 3 times 20 seconds.

b) Antibodies

b_1) Serum from a Patient Suffering from Atypical Pneumopathy

[0443] The serum designated by a reference at the National Reference Center for Influenza Viruses (Northern region) under the No. 20033168 is that from a French patient suffering from atypical pneumopathy caused by SARS-CoV collected on day 38 after the onset of the symptoms; the diagnosis of SARS-CoV infection was performed by nested RT-PCR and quantitative PCR.

b_2) Monospecific Rabbit Polyclonal Sera Directed Against the N Protein or the S Protein

[0444] The sera are those produced from the recombinant N and S_C proteins (example 2), according to the immunization protocol described in example 4; they are the rabbit P13097 serum (anti-N serum) and the rabbit P11135 serum (anti-S serum).

2) Method

[0445] 20 μl of lysate of cells infected with SARS-CoV at M.O.I. values of 10^{-1} and 10^{-2} and, as a control, 20 μl of a lysate of noninfected cells (mock) were separated on 10% SDS polyacrylamide gel and then transferred onto a nitrocellulose membrane. After blocking in a solution of PBS/5% milk/0.1% Tween and washing in PBS/0.1% Tween, this membrane was hybridized overnight at 4° C. with: (i) the immune serum No. 20033168 diluted $\frac{1}{300}$, $\frac{1}{1000}$ and $\frac{1}{3000}$ in the buffer PBS/1% BSA/0.1% Tween, (ii) the rabbit P13097 serum (anti-N serum) diluted $\frac{1}{30\,000}$ in the same buffer and (iii) the rabbit P11135 serum (anti-S serum) diluted $\frac{1}{10\,000}$ in the same buffer. After washing in PBS/Tween, a secondary hybridization was performed with the aid of either sheep polyclonal antibodies directed against the heavy and light chains of human G immunoglobulins and coupled with peroxidase (NA933V, Amersham), or of donkey polyclonal antibodies directed against the heavy and light chains of the rabbit G immunoglobulins and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized with the aid of the ECL+ kit (Amersham) and of Hyperfilm MP autoradiography films (Amersham). A molecular mass ladder (kDa) is presented in the figure.

3) Results

[0446] FIG. **7** shows that three polypeptides of apparent molecular mass 35, 55 and 200 kDa are specifically detected in the extracts of cells infected with SARS-CoV.

[0447] In order to identify these polypeptides, two other immunoblots (FIG. 8) were prepared on the same samples and under the same conditions with rabbit polyclonal antibodies specific for the nucleoprotein N (rabbit P13097, FIG. 8A) and for the spicule protein S (rabbit P11135, FIG. 8B). This experiment shows that the 200 kDa polypeptide corresponds to the SARS-CoV spicule glycoprotein S, that the 55 kDa polypeptide corresponds to the nucleoprotein N while the 35 kDa polypeptide probably represents a truncated or degraded form of N.

[0448] The data presented in FIG. 7 therefore show that the serum 20033168 strongly reacts with N and a lot more weakly with the SARS-CoV S since the 35 and 55 kDa polypeptides are visualized in the form of intense bands for 1/300, 1/1000 and 1/3000 dilutions of the immunoserum whereas the 200 kDa polypeptide is only weakly visualized for a dilution of 1/300. It is also possible to note that no other SARS-CoV polypeptide is detected for dilutions greater than 1/300 of the serum 20033168.

[0449] This experiment indicates that the antibody response specific for the SARS-CoV N dominates the antibody responses specific for the other SARS-CoV polypeptides and in particular the antibody response directed against the S glycoprotein. It indicates an immuno-dominance of the nucleoprotein N during human infections with SARS-CoV.

EXAMPLE 4

Preparation of Monospecific Polyclonal Anti-Bodies Directed Against the SARS-Associated Coronavirus (SARS-CoV) N and S Proteins

1) Materials and Method

[0450] Three rabbits (P13097, P13081, P13031) were immunized with the purified recombinant polypeptide corresponding to the entire nucleoprotein (N), prepared according to the protocol described in example 2. After a first injection of 0.35 mg per rabbit of protein emulsified in complete Freund's adjuvant (intradermal route), the animals received 3 booster injections at 3 and then 4 weeks' interval, of 0.35 mg of recombinant protein emulsified in incomplete Freund's adjuvant.

[0451] Three rabbits (P11135, P13042, P14001) were immunized with the recombinant polypeptide corresponding to the short fragment of the S protein (S_C) produced as described in example 2. As this polypeptide is found mainly in the form of inclusion bodies in the bacterial cytoplasm, the animals received 4 intradermal injections at 3-4 weeks' interval of a preparation of inclusion bodies corresponding to 0.5 mg of recombinant protein emulsified in incomplete Freund's adjuvant. The first 3 injections were made with a preparation of inclusion bodies prepared according to the protocol described in example 2, while the fourth injection was made with a preparation of inclusion bodies which were prepared according to the protocol described in example 2 and then purified on sucrose gradient and washed in 2% Triton X100.

[0452] For each rabbit, a preimmune (p.i.) serum was prepared before the first immunization and an immune serum (I.S.) 5 weeks after the fourth immunization.

[0453] In a first instance, the reactivity of the sera was analyzed by ELISA test on preparations of recombinant proteins similar to those used for the immunizations; the ELISA tests were carried out according to the protocol and with the reagents as described in example 6.

[0454] In a second instance, the reactivity of the sera was analyzed by preparing an immunoblot (Western blot) of a lysate of cells infected with SARS-CoV, according to the protocol as described in example 3.

2) Results

[0455] The ELISA tests (FIG. 9) demonstrate that the preparations of recombinant N protein and of inclusion bodies of the short fragment of the S protein (S_C) are immunogenic in animals and that the titer of the immune sera is high (more than 1/25 000).

[0456] The immunoblot (FIG. 8) shows that the rabbit P13097 immune serum recognizes two polypeptides present in the lysates of cells infected with SARS-CoV: a polypeptide whose apparent molecular mass (50-55 kDa based on experiments) is compatible with that of the nucleo-protein N (422 residues, predicted molecular mass of 46 kDa) and a polypeptide of 35 kDa, which probably represents a truncated or degraded form of N.

[0457] This experiment also shows that the rabbit P11135 serum mainly recognizes a polypeptide whose apparent molecular mass (180-220 kDa based on experiments) is compatible with a glycosylated form of S (1255 residues, nonglycosylated polypeptide chain of 139 kDa), as well as lighter polypeptides, which probably represent truncated and/or nonglycosylated forms of S.

[0458] In conclusion, all these experiments demonstrate that the recombinant polypeptides expressed in *E. coli* and corresponding to the SARS-CoV N and S proteins make it possible to induce, in animals, polyclonal antibodies capable of recognizing the native forms of these proteins.

EXAMPLE 5

Preparation of Monospecific Polyclonal Anti-Bodies Directed Against the SARS-Associated Coronavirus (SARS-CoV) M and E Proteins

1) Analysis of the Structure of the M and E Proteins

a) E Protein

[0459] The structure of the SARS-CoV E protein (76 amino acids) was analyzed in silico, with the aid of various software packages such as signalP v1.1, NetNGlyc 1.0, THMM 1.0 and 2.0 (Krogh et al., 2001, J. Mol. Biol., 305(3):567-580) or alternatively TOPPRED (von Heijne, 1992, J. Mol. Biol. 225, 487-494). The analysis shows that this nonglycosylated polypeptide is a type 1 membrane protein, containing a single transmembrane helix (aa 12-34 according to THMM), and in which the majority of the hydrophilic domain (42 residues) is located at the C-terminal end and probably inside the viral particle (endodomain). It is possible to note an inversion in the topology predicted by versions 1.0 (N-ter is external) and 2.0 (N-ter is internal) of the THMM software, but that other algorithms, in particular TOPPRED and THUMBUP (Zhou et Zhou, 2003, Protein Science 12:1547-1555) confirm an external location of the N-terminal end of E.

b) M Protein

[0460] A similar analysis carried out on the SARS-CoV M protein (221 amino acids) shows that this polypeptide does not possess a signal peptide (according to the software signalP v1.1) but three transmembrane domains (residues 15-37, 50-72, 77-99 according to THMM 2.0) and a large hydrophilic domain (aa 100-221) located inside the viral particle (endodomain). It is probably glycosylated on the asparagine at position 4 (according to NetNGlyc 1.0).

[0461] Thus, in agreement with the experimental data known for the other coronaviruses, it is remarkable that the two M and E proteins exhibit endodomains corresponding to the majority of the polypeptides and of the ectodomains that are very small in size.

[0462] The ectodomain of E probably corresponds to residues 1 to 11 or 1 to 12 of the protein: MYSFV-SEETGT(L), SEQ ID NO: 70. Indeed, the probability associated with the transmembrane location of residue 12 is intermediate (0.56 according to THMM 2.0).

[0463] The ectodomain of M probably corresponds to residues 2 to 14 of the protein: ADNGTITVEELKQ, SEQ ID NO: 69. Indeed, the N-terminal methionine of M is very probably cleaved from the mature polypeptide because the residue at position 2 is an alanine (Varshavsky, 1996, 93:12142-12149).

[0464] Moreover, the analysis of the hydrophobicity (Kyte & Doolittle, Hopp & Woods) of the E protein demonstrates that the C-terminal end of the endodomain of E is hydrophilic and therefore probably exposed at the surface of this domain. Thus, a synthetic peptide corresponding to this end is a good immunogenic candidate for inducing, in animals, antibodies directed against the endodomain of E. Consequently, a peptide corresponding to 24 C-terminal residues of E was synthesized.

2) Preparation of Antibodies Directed Against the Ectodomain of the M and E Proteins and the Endodomain of the E Protein

[0465] The peptides M2-14 (ADNGTITVEELKQ, SEQ ID NO: 69), E1-12 (MYSFVSEETGTL, SEQ ID NO: 70) and E53-76 (KPTVYVYSRV KNLNSSEGVP DLLV, SEQ ID NO: 71) were synthesized by Neosystem. They were coupled with KLH (Keyhole Limpet Hemocyanin) with the aid of MBS (m-maleimido-benzoyl-N-hydroxysuccinimide ester) via a cysteine added during the synthesis either at the N-terminus of the peptide (case for E53-76) or at the C-terminus (case of M2-14 and E1-12).

[0466] Two rabbits were immunized with each of the conjugates, according to the following immunization protocol: after a first injection of 0.5 mg of peptide coupled with KLH and emulsified in complete Freund's adjuvant (intradermal route), the animals receive 2 to 4 booster injections at 3 or 4 weeks' interval of 0.25 mg of peptide coupled to KLH and emulsified in incomplete Freund's adjuvant.

[0467] For each rabbit, a preimmune (p.i.) serum was prepared before the first immunization and an immune serum (I.S.) is prepared 3 to 5 weeks after the booster injections.

[0468] The reactivity of the sera was analyzed by Western blotting with the aid of extracts of cells infected with SARS-CoV (FIG. 43B) or with the aid of extracts of cells infected with a recombinant vaccinia virus expressing the protein E (VV-TG-E, FIG. 43A) or M (VV-TN-M, FIG. 43C) of the SARS-CoV 031589 isolate.

[0469] The immune sera of the rabbits 22234 and 22240, immunized with the conjugate KLH-E53-76, recognize a polypeptide of about 9 to 10 kD, which is present in the extracts of cells infected with SARS-CoV but absent from the extracts of noninfected cells (FIG. 43B). The apparent mass of this polypeptide is compatible with the predicted mass of the E protein, which is 8.4 kD. Similarly, the immune serum of the rabbit 20047, immunized with the conjugate KLH-E1-12, recognizes a polypeptide present in the extracts of cells infected with the VV-TG-E virus, whose apparent molar mass is compatible with that of the E protein (FIG. 43A).

[0470] The immune serum of the rabbits 20013 and 20080, immunized with the conjugate KLH-M2-14, recognizes a polypeptide present in the extracts of cells infected with the VV-TN-M virus (FIG. 43C), whose apparent molar mass (about 18 kD) is compatible with that of the glycoprotein M, which is 25.1 kD and has a high iso-electric point (9.1 for the naked polypeptide).

[0471] These results demonstrate that the peptides E1-12 and E53-76, on the one hand, and the peptide M2-14, on the other hand, make it possible to induce, in animals, polyclonal antibodies capable of recognizing the native forms of the SARS-CoV E and M proteins, respectively.

EXAMPLE 6

Analysis of the ELISA Reactivity of the Recombinant N Protein Toward Sera from Patients Suffering from SARS

1) Materials

[0472] The antigen used to prepare the solid phases is the purified recombinant nucleoprotein N prepared according to the protocol described in example 2.

[0473] The sera to be tested (table IV) were chosen on the basis of the results of analysis of their reactivity by immunofluorescence (IF-SARS titer), toward cells infected with SARS-CoV.

TABLE IV

Sera tested by ELISA

Reference	Serum No.	Type of serum	Date of the serum***	IF-SARS titer
3050	A	Control	na*	nt**
3048	B	Control	na	nt
033168	D	Patient 1-SARS	Apr. 27, 2003 (D38)	320
033397	E	Patient-1 SARS	May 11, 2005 (D52)	320
032632	F	Patient-2 SARS	Mar. 21, 2003 (D17)	2500
032791	G	Patient-3 SARS	Apr. 04, 2003 (D3)	<40
033258	H	Patient-3 SARS	Apr. 28, 2003 (D27)	160

*na: not applicable.
**nt: not tested.
***the dates indicated correspond to the number of days after the onset of the SARS symptoms.

2) Method

[0474] The N protein (100 µl) diluted at various concentrations in 0.1 M carbonate buffer, pH 9.6 (1, 2 or 4 µg/ml) is distributed into the wells of ELISA plates, and then the plates are incubated overnight at laboratory temperature. The plates are washed with PBS-Tween buffer saturated with PBS-skimmed milk-sucrose (5%) buffer. The test sera (100 µl), diluted beforehand (1/50, 1/100, 1/200, 1/400, 1/800, 1/1600 and 1/3200) are added and then the plates are incubated for 1 h at 37° C. After 3 washings, the peroxidase-labeled anti-human IgG conjugate (reference 209-035-098, JACKSON) diluted 1/18 000 is added and then the plates are incubated for 1 h at 37° C. After 4 washings, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 30 min at room temperature, protected from light. The reaction is then stopped and then the absorbance at 450 nm is measured with the aid of an automated reader.

3) Results

[0475] The ELISA tests (FIG. **10**) demonstrate that the recombinant N protein preparation is specifically recognized by the antibodies of sera from patients suffering from SARS collected in the late phase of the infection (≧17 days after the onset of the symptoms) whereas it is not significantly recognized by the antibodies of a patient's serum collected in the early phase of the infection (3 days after the onset of the symptoms) or by control sera from subjects not suffering from SARS.

EXAMPLE 7

ELISA Tests Prepared for a Very Specific and Sensitive Detection of a SARS-Associated Coronavirus Infection, from Sera of Patients

1) Indirect ELISA IgG Test

a) Reagents

Preparation of the Plates

[0476] The plates are sensitized with a solution of N protein at 2 µg/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l. 100 µl of solution are deposited in the wells and left to incubate at room temperature overnight. Saturation is obtained by prewashing in 10 mM PBS/0.1% Tween buffer, followed by washing with a saturation solution PBS, 25% milk/sucrose.

Diluent Sera

[0477] Buffer 0.48 g/l TRIS, 10 mM PBS, 3.7 g/l EDTA, 15% v/v milk, pH 6.7

Diluent Conjugate

[0478] Citrate buffer (15 g/l), 0.5% Tween, 25% bovine serum, 12% NaCl, 6% v/v skimmed milk pH 6.5

Conjugate

[0479] 50× anti-human IgG conjugate, marketed by Bio-Rad: Platelia *H. pylori* kit ref 72778

Other Solutions:

[0480] Washing solution R2, solutions for visualizing with TMB R8 diluent, R9 chromogen, R10 stopping solution: reagents marketed by Bio-Rad (e.g.: *Platelia pylori* kit, ref 72778)

b) Procedure

[0481] Dilute the sera 1/200 in the sample diluent

[0482] Distribute 100 µl/well

[0483] Incubation 1 h at 37° C.

[0484] 3 washings in 10× WASHING solution R2 diluted before-hand 10-fold in demineralized water (i.e., 1× washing solution)

[0485] Distribute 100 µl of conjugate (50× conjugate to be diluted immediately before use in the diluent conjugate provided)

[0486] Incubation 1 h at 37° C.

[0487] 4 washings in 1× washing solution

[0488] Distribute 200 µl/well of visualization solution (to be diluted immediately before use e.g.: 1 ml of R9 in 10 ml of R8)

[0489] Incubation for 30 min at room temperature in the dark

[0490] Stop the reaction with 100 µl/well of R10

[0491] READING at 450/620 nm

[0492] The results can be interpreted by taking a THRESHOLD serum giving a response above which the sera tested would be considered as positive. This serum is chosen and diluted so as to give a significantly higher signal than the background noise.

2) Double Epitope Elisa Test

a) Reagents

Preparation of the Plates

[0493] The plates are sensitized with a solution of N protein at 1 g/ml in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l. 100 µl of solution are deposited in the wells and left to incubate at room temperature overnight. Saturation is obtained by prewashing in 10 mM PBS/0.1% Tween buffer, followed by washing with a saturation solution 10 mM PBS, 25% (V/V) milk.

Diluent Sera and Conjugate

[0494] Buffer 50 mM TRIS saline, pH 8, 2% milk

Conjugate

[0495] This is the purified recombinant N protein coupled with peroxidase according to the Nakane protocol (Nakane P. K. and Kawaoi A.; (1974): *Peroxydase-labeled antibody, a new method of conjugation. The Journal of Histochemistry and Cytochemistry* Vol. 22, N) 23, pp. 1084-1091), in respective molar ratios 1/2. This ProtN POD conjugate is used at a concentration of 2 µg/ml in serum/conjugate diluent.

Other Solutions:

[0496] Washing solution R2, solutions for visualization with TMB R8, diluent, R9 chromogen, R10 stopping solution: reagents marketed by Bio-Rad (e.g. *Platelia pylori* kit, ref 72778).

b) Procedure

[0497] 1st step in "predilution" plate

[0498] Dilute each serum ⅕ in the predilution plate

[0499] (48 µl of diluent+12 µl of serum).

[0500] After having diluted all the sera, distribute 60 µl of conjugate.

[0501] Where appropriate, the serum+conjugate mix is left to incubate.

[0502] 2nd step in "reaction" plate

[0503] Transfer 100 µl of mixture/well into the reaction plate

[0504] Incubation 1 h 37° C.

[0505] 5 washings in 10× WASHING solution R2 diluted 10-fold beforehand in demineralized water (→1× washing solution)

[0506] Distribute 200 µl/well of visualization solution (to be diluted immediately before use e.g.: 1 ml of R9 in 10 ml of R8)

[0507] Incubation 30 min at room temperature and protected from light

[0508] Stop the reaction with 100 µl/well of R10

[0509] READING at 450/620 nm

[0510] Likewise as for the indirect ELISA test, the results can be interpreted using a "threshold value" serum. Any serum having a response greater than the threshold value serum will be considered as positive.

2) Results

[0511] The sera of patients classified as probable cases of SARS from the French hospital of Hanoi, Vietnam or in relation with the French hospital of Hanoi (JYK) were analyzed using the indirect IgG-N test and the double epitope N test.

[0512] The results of the indirect IgG-N test (FIGS. **14** and **15**) and double epitope N test (FIGS. **16** and **17**) show an excellent correlation between them and with an indirect ELISA test comparing the reactivity of the sera toward a lysate of VeroE6 cells infected or not infected with SARS-CoV (ELISA-SARS-CoV lysate; see table V below). All the sera collected 12 days or more after the onset of the symptoms were found to be positive, including in patients for whom it had not been possible to document the SARS-CoV virus infection by analyzing respiratory samples by RT-PCR, probably because of a sample being collected too late during the infection (≧D12). In the case of the patient TTH for whom a nasal sample collected on D7 was found to be negative by RT-PCR, the quality of the sample may be in question.

[0513] Some sera were found to be negative whereas the presence of SARS-CoV was detected by RT-PCR. They are in all cases early sera collected less than 10 days after the onset of the symptoms (e.g.: serum # 032637). In the case of a patient PTTH (serum # 032673), only a suspicion of SARS was raised at the time the samples were collected.

[0514] In conclusion, the indirect IgG-N and N-double epitope serological tests make it possible to document the SARS-CoV infection in all the patients for the sera collected 12 days or more after the infection.

TABLE V

Results of the ELISA tests

Sample Num	Patient	Day	PCR-SARS (1)	ELISA SARS-CoV lysate (2)	IgG-N (2nd series)	2Xepitope (2nd series)
033168	JYK	38	POS	+++	>5000	NT
033597	JYK	74	POS	NT	≈5000	NT
032552	VTT	8	NEG-D3&D8&D12	NEG	<200	<5
032544	CTP	16	NEG D16&D20	++	>5000	>>20
032546	CJF	15	NEG D15&D19	++	>5000	>>20
032548	PTL	17	NEG D17&D21	++	>5000	>>20
032550	NTH	17	NEG-D17&D21	++	>5000	>>20
032553	VTT	8	NEG-D3&D8&D12	NEG	<200	<5
032554	NTBV	4	POS	NEG	<200	<5
032555	NTBV	4	POS	NEG	<200	
032564	NTP	15	POS	++	>5000	>>20
032629	NVH	4	POS	NEG	<200	<5
032631	BTTX	9	POS	NEG	<200	<5
032635	NHH	4	POS	NEG	<200	<5
032637	NHB	10	POS	NEG	<200	<5
032642	BTTX	9	POS	NEG	<200	<5
032643	LTDH	1	POS	NEG	<200	<5
032644	NTBV	4	POS	NEG	<200	<5
032646	TTH	12	NEG D7&D12&D16	++	>5000	>>20
032647	DTH	17	NEG D17&D21	++	>5000	>>20

TABLE V-continued

Results of the ELISA tests

Sample Num	Patient	Day	PCR-SARS (1)	ELISA SARS-CoV lysate (2)	IgG-N (2nd series)	2Xepitope (2nd series)
032648	NNT	15	NEG D15&D19	++	>5000	>>20
032649	PTH	17	NEG D17&D21	++	>5000	>>20
032672	LVV	16	NEG D16&D20	+	>5000	>>20
032673	PTTH	NA	NEG	NEG	<200	<5
032674	PNB	17	NEG D17&D21	++	>5000	>>20
032682	VTH	12	NEG D12&D16	++	>5000	>>20
032683	DTV	17	NEG D17&D21	+	>1000	>>20

Remarks:

[0515] (1): The RT-PCR analyses were carried out by nested RT-PCR BNI, LC Artus and LC-N on nasal or pharyngeal swabs; POS means that at least one sample was found to be positive in this patient.

[0516] (2): The reactivity of the sera in the ELISA test using a lysate of cells infected with SARS-CoV was classified as very highly reactive (+++), highly reactive (++), reactive (+) and negative according to the OD value obtained at the dilutions tested.

EXAMPLE 8

Detection of SARS-Associated Coronavirus (SARS-CoV) by RT-PCR

1) Real Time Development of RT-PCR Conditions with the Aid of Primers Specific for the Gene for the Nucleocapsid Protein—"Light Cycler N" Test

a) Design of the Primers and Probes

[0517] The primers and probes were designed from the sequence of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589, with the aid of the programme "Light Cycler Probe Design (Roche)". Thus, the following two series of primers and probes were selected:

```
series 1 (SEQ ID NO: 60, 61, 64, 65):
sense primer: N/+/28507:
5'-GGC ATC GTA TGG GTT G-3'
[28507-28522]

antisense primer: N/-/28774:
5'-CAG TTT CAC CAC CTC C-3'
[28774-28759]

probe 1:
5'-GGC ACC CGC AAT CCT AAT AAC AAT GC-
fluorescein 3'
[28561-28586]

probe 2:
5' Red705-GCC ACC GTG CTA CAA CTT CCT-phosphate
[28588-28608]
```

-continued

```
series 2 (SEQ ID NO: 62, 63, 66, 67)
sense primer: N/+/28375:
5'-GGC TAC TAC CGA AGA G-3'
[28375-28390]

antisense primer: N/-/28702:
5'-AAT TAC CGC GAC TAC G-3'
[28702-28687]

probe 1: SARS/N/FL:
5'-ATA CAC CCA AAG ACC ACA TTG GC-fluorescein 3'
[28541-28563]

probe 2: SARS/N/LC705:
5' Red705-CCC GCA ATC CTA ATA ACA ATG CTG C-
phosphate 3'
[28565-28589]
```

b) Analysis of the Efficacy of the Two Primer Pairs

[0518] In order to test the respective efficacy of the two pairs of primers, an RT-PCR amplification was carried out on a synthetic RNA corresponding to nucleotides 28054-29430 of the genome of the SARS-CoV strain derived from the sample recorded under the number 031589 and containing the sequence of the N gene.

[0519] More specifically:

[0520] This synthetic RNA was prepared by in vitro transcription with the aid of the T7 phage RNA polymerase, of a DNA template obtained by linearization of the plasmid SRAS-N with the enzyme Bam H1. After eliminating the DNA template by digestion with the aid of DNAse 1, the synthetic RNAs are purified by a phenol-chloroform extraction, followed by two successive precipitations in ammonium acetate and isopropanol. They are then quantified by measuring the absorbance at 260 nm and their quality is checked by the ratio of the absorbances at 260 and 280 nm and by agarose gel electrophoresis. Thus, the concentration of the synthetic RNA preparation used for these studies is 1.6 mg/ml, which corresponds to 2.1×10^{15} copies/ml of RNA.

[0521] Decreasing quantities of synthetic RNA were amplified by RT-PCR with the aid of the "Superscript™

One-Step RT-PCR with Platinum® Taq" kit and the pairs of primers No. 1 (N/+/28507, N/−/28774) (FIG. 1A) and No. 2 (N/+/28375, N/−/28702) (FIG. 1B), according to the supplier's instructions. The amplification conditions used are the following: the cDNA was synthesized by incubation for 30 min at 45° C., 15 min at 55° C. and then 2 min at 94° C. and it was then amplified by 5 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 45° C. for 30 sec and then a step of extension at 72° C. for 30 sec, followed by 35 cycles comprising: a step of denaturation at 94° C. for 15 sec, a step of annealing at 55° C. for 30 sec and then a step of extension at 72° C. for 30 sec, with 2 sec of additional extension at each cycle, and a final step of extension at 72° C. for 5 min. The amplification products obtained were then kept at 10° C.

[0522] The results presented in FIG. 11 show that the pair of primers No. 2 (N/+/28375, N/−/28702) makes it possible to detect up to 10 copies of RNA (band of weak intensity) or 10^2 copies (band of good intensity) against 10^4 copies for the pair of primers No. 1 (N/+/28507, N/−/28774). The amplicons are respectively 268 bp (pair 1) and 328 bp (pair 2).

c) Development of Real Time RT-PCR

[0523] A real time RT-PCR was developed with the aid of the pair of primers No. 2 and of the pair of probes consisting of SRAS/N/FL and SRAS/N/LC705 (FIG. 2).

[0524] The amplification was carried out on a LightCycler™ (Roche) with the aid of the "Light Cycler RNA Amplification Kit Hybridization Probes" kit (reference 2 015 145, Roche) under the following optimized conditions. A reaction mixture containing: H_2O (6.8 μl), 25 mM $MgCl_2$ (0.8 μl, 4 μM Mg2+ final), 5× reaction mixture (4 μl), 3 μm probe SRAS/N/FL (0.5 μl, 0.075 μM final), 3 μM probe SRAS/N/LC705 (0.5 μl, 0.075 μM final), 10 μM primer N/+/28375 (1 μl, 0.5 μM final), 10 μM primer N/−/28702 (1 μl, 0.5 μM final), enzyme mixture (0.4 μl) and sample (viral RNA, 5 μl) was amplified according to the following program:

Reverse transcription:	50° C.	10:00 min	analysis mode: none	
Denaturation:	95° C.	30 sec × 1	analysis mode: none	
Amplification:	95° C.	2 sec		
	50° C.	15 sec	analysis mode: quantification*	×45
	72° C.	13 sec	thermal ramp 2.0° C./sec	
Annealing:	40° C.	30 sec × 1	analysis mode: none	

*The fluorescence is measured at the end of the annealing and at each cycle (in SINGLE mode).

[0525] The results presented in FIG. 12 show that this real time RT-PCR is very sensitive since it makes it possible to detect 102 copies of synthetic RNA in 100% of the 5 samples analyzed (29/29 samples in 8 experiments) and up to 10 copies of RNA in 100% of the 5 samples analyzed (40/45 samples in 8 experiments). It also shows that this RT-PCR makes it possible to detect the presence of the SARS-CoV genome in a sample and to quantify the number of genomes present. By way of example, the viral RNA of a SARS-CoV stock cultured on Vero E6 cells was extracted with the aid of the "Qiamp viral RNA extraction" kit (Qiagen), diluted to 0.05×10^{-14} and analyzed by real time RT-PCR according to the protocol described above; the analysis presented in FIG. 12 shows that this virus stock contains 6.5×10^9 genome-equivalents/ml (geq/ml), which is entirely similar to the 1.0×10^{10} geq/ml value measured with the aid of the "RealArt™ HPA-Coronavirus LC RT PCR Reagents" kit marketed by Artus.

2) Development of Nested RT-PCR Conditions Targeting the Gene for RNA Polymerase—"CDC (Centers for Disease Control and Prevention)/IP Nested RT-PCR" Test

a) Extraction of the Viral RNA

[0526] Clinical sample: QIAmp viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions, or an equivalent technique. The RNA is eluted in a volume of 60 μl.

b) "SNE/SAR" Nested RT-PCR

First Step: "SNE" Coupled RT-PCR

[0527] The Invitrogen "Superscript™ One-Step RT-PCR with Platinum® Taq" kit was used, but the "Titan" kit from Roche Boehringer can be used in its place with similar results.

```
Oligonucleotides:
SNE-S1
5' GGT TGG GAT TAT CCA AAA TGT GA 3'

SNE-AS1
5' GCA TCA TCA GAA AGA ATC ATC ATG 3'

→ Expected size: 440 bp
```

[0528] 1. Prepare a mix:

H2O	6.5 μl
Reaction mix 2X	12.5 μl
Oligo SNE-S1 50 μM	0.2 μl

-continued

Oligo SNE-AS1 50 μM	0.2 μl
RNAsin 40 U/μl	0.12 μl
RT/Platinum Taq mix	0.5 μl

[0529] 2. To 20 μl of the mix, add 5 μl of RNA and carry out the amplification on a thermocycler (ABI 9600 conditions):

2.1	45° C.	30 min.	
	55° C.	15 min.	
	94° C.	2 min.	
2.2.	94° C.	15 sec.	} ×5 cycles
	45° C.	30 sec.	
	72° C.	30 sec.	
2.3.	94° C.	15 sec.	} ×35 cycles
	55° C.	30 sec.	
	72° C.	30 sec. + 2 sec./cycle	
2.4.	72° C.	5 min.	
2.5	10° C.	∞	

Storage at +4° C..

[0530] The RNAsin (N2511/N2515) from Promega was used as RNase inhibitors.

[0531] Synthetic RNAs served as positive control. As the control, 10^3, 10^2 and 10 copies of synthetic RNA R_{SNE} were amplified in each experiment.

[0532] Second Step: "SAR" Nested PCR

```
Oligonucleotides:
SAR1-S
5' CCT CTC TTG TTC TTG CTC GCA 3'

SAR1-AS
5' TAT AGT GAG CCG CCA CAC ATG 3'

→ Expected size: 121 bp
```

[0533] 1. Prepare a mix:

H2O	35.8 µl
Taq buffer 10X	5 µl
$MgCl_2$ 25 mM	4 µl
Mix dNTPs 5 mM	2 µl
Oligo SAR1-S 50 µM	0.5 µl
Oligo SAR1-AS 50 µM	0.5 µl
Taq DNA pol 5 U/µl	0.25 µl

[0534] AmpliTaq DNA Pol from Applied Biosystems was used (10× buffer without $MgCl_2$, ref 27216601).

[0535] 2. To 48 µl of the mix, add 2 µl of the product from the first PCR and carry out the amplification (ABI 9600 conditions):

2.1.	94° C.	2 min.	
2.2.	94° C.	30 sec.	} ×5 cycles
	45° C.	45 sec.	
	72° C.	30 sec.	
2.3.	94° C.	30 sec.	} ×35 cycles
	55° C.	30 sec.	
	72° C.	30 sec. + 1 sec./cycle	
2.4.	72° C.	5 min.	
2.5	10° C.	∞	

[0536] 3. Analyze 10 µl of the reaction product on "low-melting" gel (Seakem GTG type) containing 3% agarose.

[0537] The sensitivity of the nested test is routinely, under the conditions described, 10 copies of RNA.

[0538] 4. The fragments can then be purified on QIAquick PCR kit (QIAGEN) and sequenced with the oligos SAR1-S and SAR1-AS.

3) Detection of the SARS-CoV RNA by PCR from Respiratory Samples

a) First Comparative Study

[0539] A comparative study was carried out on a series of respiratory samples received by the National Reference Center for the Influenza Virus (Northern region) and likely to contain SARS-CoV. To do this, the RNA was extracted from the samples with the aid of the "Qiamp viral RNA extraction" kit (Qiagen) and analyzed by real time RT-PCR, on the one hand with the aid of the pairs of primers and probes of the No. 2 series under the conditions described above on the one hand, and on the other hand with the aid of the kit "LightCycler SARS-CoV quantification kit" marketed by Roche (reference 03 604 438). The results are summarized in table VI below. They show that 18 of the 26 samples are negative and 5 of the 26 samples are positive for the two kits, while one sample is positive for the Roche kit alone and two for the "series 2" N reagents alone., Additionally, for 3 samples (20032701, 20032712, 20032714) the quantities of RNA detected are markedly higher with the reagents (probes and primers) of the No. 2 series. These results indicate that the "series 2" N primers and probes are more sensitive for the detection of the SARS-CoV genome in biological samples than those of the kit currently available.

TABLE VI

Real time RT-PCR analysis of the RNAs extracted from a series of samples from 5 patients with the aid of the pairs of primers and probes of the No. 2 series ("series 2" N) or of the kit "Lightcycler SARS-CoV quantification kit" (Roche). The type of sample is indicated as well as the number of copies of viral genome measured in each of the two tests. NEG: negative RT-PCR.

Sample No.	Patient	Type of sample	ROCHE KIT	"Series 2" N
20033082	K	nasal	NEG	NEG
20033083	K	pharyngeal	NEG	NEG
20033086	K	nasal	NEG	NEG
20033087	K	pharyngeal	NEG	NEG
20032802	M	nasal	NEG	NEG
20032803	M	expectoration	NEG	NEG
20032806	M	nasal or pharyngeal	NEG	NEG
20031746ARN2	C	pharyngeal	NEG	NEG
20032711	C	nasal or pharyngeal	39	NEG
20032910	B	nasal	NEG	NEG
20032911	B	pharyngeal	NEG	NEG
20033356	V	expectoration	NEG	NEG
20033357	V	expectoration	NEG	NEG
20031725	K	endotracheal asp.	NEG	150
20032657	K	endotracheal asp.	NEG	NEG
20032698	K	endotracheal asp.	NEG	NEG
20032720	K	endotracheal asp.	3	5
20033074	K	stools	115	257
20032701	M	pharyngeal	443	1676
20032702	M	expectoration	NEG	249
20031747ARN2	C	pharyngeal	NEG	NEG
20032712	C	unknown	634	6914
20032714	C	pharyngeal	17	223
20032800	B	nasal	NEG	NEG
20033353	V	nasal	NEG	NEG
20033384	V	nasal	NEG	NEG

b) Second Comparative Study

[0540] The performance of various nested RT-PCR and real time RT-PCR methods were then compared for 121 respiratory samples from possible cases of SARS at the French hospital in Hanoi, Vietnam, taken between the 4th and the 17th day after the onset of the symptoms. Among these samples, 14 were found to be positive during a first test using the nested RT-PCR method targeting ORF1b (encoding replicase) as described initially by Bernhard Nocht Institute (BNI nested RT-PCR). Information relating to this test is available on the internet, at the address http://www15.bni-hamburg.de/bni2/neu2/getfile.acgi?area_eng1=diagnostics&pid=4112.

[0541] The various tests compared in this study are:

[0542] the quantitative RT-PCR method according to the invention, with the "series 2" N primers and probes described above (LightCycler N column),

[0543] the nested RT-PCR test targeting the RNA polymerase gene described above, developed by the CDC, BNI and Institut Pasteur (CDC/IP nested RT-PCR),

[0544] the ARTUS kit with the reference "HPA Corona LC RT-PCR Kit # 5601-02", which is a real time RT-PCR test targeting the ORF1b gene,

[0545] the BNI nested RT-PCR test, also targeting the RNA polymerase gene mentioned above.

[0546] The inventors observed:

[0547] 1) an inter-test variability for the same technique, linked to the degradation of the RNA preparation during repeated thawing, in particular for the samples containing the lowest quantities of RNA,

[0548] 2) a reduced sensitivity of the CDC/IP nested RT-PCR compared with the BNI nested RT-PCR, and

[0549] 3) a comparable sensitivity of the quantitative RT-PCR test according to the invention (LightCycler N) compared with the Artus LightCycler (LC) test.

[0550] These results, which are presented in table VII below, show that the quantitative RT-PCR test according to the invention constitutes an excellent addition—or an alternative—to the tests currently available. Indeed, the SARS-linked coronavirus is an emergent virus which is capable of changing rapidly. In particular, the gene for the RNA polymerase of the SARS-linked coronavirus, which is targeted in most of the tests currently available, can recombine with that of other coronaviruses not linked to SARS. The use of a test targeting this gene exclusively could then lead to the production of false-negatives.

[0551] The quantitative RT-PCR test according to the invention does not target the same genomic region as the ARTUS kit since it targets the gene encoding the N protein. By carrying out a diagnostic test targeting two different genes of the SARS-linked coronavirus, it can therefore be hoped to avoid false-negative type results which could be due to the genetic evolution of the virus.

[0552] Furthermore, it appears particularly advantageous to target the gene for the nucleocapsid protein because it is very stable because of the high selection pressure linked to the high structural constraints regarding this protein.

TABLE VII

Comparison of various methods of analysis by gene amplification, from 121 samples of probable cases of SARS at the French hospital in Hanoi, Vietnam (epidemic 2003)

NRC No.	Sample type (1)	Sample collection day	Patient	CDC/IP nested RT-PCR	BNI nested RT-PCR	Artus Light Cycler kit	Light Cycler N (IP)
107 samples	N and P			Negative	Negative	Negative	Negative
032529	P	10	NHB	Negative	Positive	Negative	Negative
032530	N	10	NHB	Positive	Positive	3.10E+01	4.20E+01
032531	P	7	LP	Positive	Positive	7.70E+00	3.10E+00
032534	N	15	BND	Positive	Positive	1.60E+00	Negative
032600	P	4	NHH	Negative	Positive	Negative	0.30E+02
032612	P	17	NTS	Negative	Positive	Negative	Negative
032688	P	9	BTX	Positive	Positive	Negative	Negative
032689	N	4	NVH	Positive	Positive	1.20E+01	2.30E+02
032690	P	4	NVH	Negative	Positive	1.60E+00	Negative
032727	P	8	NVH	Positive	Positive	2.30E+02	4.00E+02
032728	N	8	NVH	Positive	Positive	1.10E+03	1.60E+04
032729	P	14	NHB	Positive	Positive	5.90E+00	3.40E+01
032730	N	14	NHB	Positive	Positive	1.30E+02	4.80E+02
032741	P	8	NHH	Positive	Positive	2.10E+02	1.30E+02
positives				10	14	10	9
fraction detected from the 14 positives				71.4%	100.0%	71.4%	64.3%

(1) P = pharyngeal swab N = nasal swab

EXAMPLE 9

Production and Characterization of Monoclonol Antibodies Directed Against the N Protein

[0553] Balb C mice were immunized with the purified recombinant N protein and their spleen cells fused with an appropriate murine myeloma according to the Köhler and Milstein techniques.

[0554] Nineteen anti-N antibody secreting hybridomas were preselected and their immunoreactivities determined. These antibodies do indeed recognize the recombinant N protein (in ELISA) with variable intensities, and the natural viral N protein in ELISA and/or in Western blotting. FIGS. **18** to **20** show the results of these tests for 15 of these 19 monoclonal antibodies.

[0555] The highly reactive clones 12, 17, 28, 57, 72, 76, 86, 87, 98, 103, 146, 156, 166, 170, 199, 212, 218, 219 and 222 were subcloned. Specificity studies were carried out with the appropriate tools in order to determine the epitopes recognized and verify the absence of reactivity toward other human coronaviruses and certain respiratory viruses.

[0556] Epitope mapping studies (performed on spot membrane with the aid of overlapping peptides of 15 aa) and additional studies performed on the natural N protein in Western blotting revealed the existence of 4 groups of monoclonal antibodies:

[0557] 1. Monoclonal antibodies specific for a major linear epitope at the N-ter position (75-81, sequence: INTNSVP).

[0558] The representative of this group is antibody 156. The hybridoma producing this antibody was deposited at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institut Pasteur (Paris, France) on Dec. 1, 2004, under the number I-3331. This same epitope is also recognized by a rabbit serum (anti-N polyclonal) obtained by conventional immunization with the aid of this same N protein.

[0559] 2. Monoclonal antibodies specific for a major linear epitope located in a central position (position 217-224, sequence: ETALALL); the representatives of this group are the monoclonal antibodies 87 and 166. The hybridoma producing antibody 87 was deposited at the CNCM on Dec. 1, 2004, under the number I-3328.

[0560] 3. Monoclonal antibodies specific for a major linear epitope located at the C-terminal position (position 403-408, sequence: DFFRQL), the representatives of this group are the antibodies 28, 57 and 143. The hybridoma producing antibody 57 was deposited at the CNCM on Dec. 1, 2004, under the number I-3330.

[0561] 4. Monoclonal antibodies specific for a discontinuous conformational epitope. This group of antibodies does not recognize any of the peptides spanning the sequence of the N protein, but react strongly on the non-denatured natural protein. The representative of this final group is the antibody 86. The hybridoma producing this antibody was deposited at the CNCM on Dec. 1, 2004, under the number I-3329.

[0562] Table VIII below summarizes the epitope mapping results obtained:

TABLE VIII

	Epitope mapping of the monoclonal antibodies		
Antibody	Epitope	Position	Region
28	**DFSRQL** *Q*	403 . . . 408	C-Ter.
143	**DFSRQL** *Q*		
76	**DFSRQL** *Q*		
57	**DFSRQL** *Q*		
	FFGMS RI	315 . . . 319	
146	**LPQRQ**	383 . . . 387	
166	ETALALL*LL*	217 . . . 224	central
87	**ETALALL**	217 . . . 224	
156	***INTNSGP***	75 . . . 81	N-Ter.
86	Conformational		
212	Conformational		
170	Conformational		

[0563] In addition, as illustrated in particular in FIGS. **18** and **19**, these antibodies exhibit no reactivity in ELISA and/or in WB toward the N protein of the human coronavirus 229 E.

EXAMPLE 10

Combinations of the Monoclonal Antibodies for the Development of a Sensitive Immunocapture Test Specific for the Viral N Antigen in the Serum or Biological Fluids of Patients Infected with the SARS-CoV Virus

[0564] The antibodies listed below were selected because of their very specific properties for an additional capture and detection study of the viral N protein, in the serum of the subjects or patients.

[0565] These antibodies were produced in ascites on mice, purified by affinity chromatography and used alone or in combination, as capture antibodies and as signal antibodies.

[0566] List of the antibodies selected:

[0567] Ab anti-C-ter region (No. 28, 57, 143)

[0568] Ab anti-central region (No. 87, 166)

[0569] Ab anti-N-ter region (No. 156)

[0570] Ab anti-discontinuous conformational epitope (86)

1) Preparation of the Reagents:

a) Immunocapture ELISA Plates

[0571] The plates are sensitized with the antibody solutions at 5 μg/ml in 0.1 M carbonate buffer, pH 9.6. The (monovalent or plurivalent) solutions are deposited in a volume of 100 μl in the wells and incubated overnight at room temperature. These plates are then washed with PBS buffer (10 mM pH 7.4 supplemented with 0.1% Tween 20) and then saturated with a PBS solution supplemented with 0.3% BSA and 5% sucrose). The plates are then dried and then packaged in a bag in the presence of a desiccant. They are ready to use.

b) Conjugates

[0572] The purified antibodies were coupled with peroxidase according to the Nakane protocol (Nakane et al.—1974, J. of Histo and cytochemistry, vol. 22, pp. 1084-1091) in a ratio of one molecule of IgG per 3 molecules of peroxidase. These conjugates were purified by exclusion chromatography and stored concentrated (concentration between 1 and 2 mg/ml) in the presence of 50% glycerol and at −20° C. They are diluted for their use in the assays at the final concentration of 1 or 2 μg/ml in PBS buffer (pH 7.4) supplemented with 1% BSA.

c) Other Reagents

[0573] Human sera negative for all the serum markers for the HIV, HBV, HCV and THLV viruses Pool of negative human sera supplemented with 0.5% Triton X 100

[0574] Inactivated viral Ag: viral culture supernatant inactivated by irradiation and inactivation verified after placing in culture on sensitive cells—titer of the suspension before inactivation about 10^7 infectious particles per ml or alternatively about 5×10^9 physical viral particles per ml of antigen

[0575] The Ag samples diluted in negative human serum: these samples were prepared by diluting 1:100 and then by 5-fold serial dilution.

[0576] These noninfectious samples mimic human samples thought to contain low to very low concentrations of viral nucleoprotein N. Such samples are not available for routine work.

[0577] Washing solution R2, solution for visualization TMB R8, chromogen R9 and stop solution R10, are the generic reagents marketed by Bio-Rad in its ELISA kits (e.g.: *Platelia pylori* kit ref. 72778).

2) Procedure

[0578] The samples of human sera overloaded with inactivated viral Ag are distributed in an amount of 100 μl per well, directly in the ready-to-use sensitized plates, and then incubated for 1 hour at 37° C. (Bio-Rad IPS incubation).

[0579] The material not bound to the solid phase is removed by 3 washings (washing with dilute R2 solution, automatic LP 35 washer).

[0580] The appropriate conjugates, diluted to the final concentration of 1 or 2 μg/ml, are distributed in an amount of 100 μl per well and the plates are again incubated for one hour at 37° C. (IPS incubation).

[0581] The excess conjugate is removed by 4 successive washings (dilute R2 solution—LP 35 washer).

[0582] The presence of conjugate attached to the plates is visualized after adding 100 μl of visualization solution prepared before use (1 ml of R9 and 10 ml of R8) and after incubation for 30 minutes, at room temperature and protected from light.

[0583] The enzymatic reaction is finally blocked by adding 100 μl of R10 reagent (1 N H_2SO_4) to all the wells.

[0584] The reading is carried out with the aid of an appropriate microplate reader at double wavelength (450/620 nm).

[0585] The results can be interpreted by using, as provisional threshold value, the mean of at least two negative controls multiplied by a factor of 2 or alternatively the mean of 100 negative sera supplemented with an increment corresponding to 6 SD (standard deviation calculated on the 100 individual measurements).

3) Results

[0586] Various capture antibody and signal antibody combinations were tested based on the properties of the antibodies selected, and avoiding the combinations of antibodies specific for the same epitopes in solid phase and as conjugates.

[0587] The best results were obtained with the 4 combinations listed below. These results are reproduced in table IX below.

1. Combination F/28

[0588] Solid phase (Ab 166+87 central region): conjugate antibody 28 (C-ter)

2. Combination G/28

[0589] Solid phase (Ab 86—conformational epitope): conjugate antibody 28 (C-ter)

3. Combination H/28

[0590] Solid phase (Ab 86, 166 and 87 central region and conformational epitope): conjugate antibody 28 (C-ter)

4. Combination H/28+87

[0591] Solid phase (Ab 86, 166 and 87 central region and conformational epitope): mixed conjugate antibodies 28 (C-ter) and 87 (central)

5. Combination G/87

[0592] Solid phase (Ab 86—conformational epitope): conjugate antibody 87 (central region)

[0593] The first 4 combinations exhibit equivalent and reproduced performance levels, greater than the other combinations used (such as for example the combination G/87). Of course, in these combinations, a monoclonal antibody may be replaced with another antibody recognizing the same epitope. Thus, the following variants may be mentioned:

6. Variant of the Combination F/28

[0594] Solid phase (Ab 87 only): conjugate antibody 57 (C-ter)

7. Variant of the Combination G/28

[0595] Solid phase (Ab 86—conformational epitope): conjugate antibody 57 (C-ter)

8. Variant of the Combination H/28

[0596] Solid phase (Ab 86 and 87 central region and conformational epitope): conjugate antibody 57 (C-ter)

9. Variant of the Combination H/28+87

[0597] Solid phase (Ab 86 and 87 central region and conformational epitope): mixed conjugate antibodies 57 (C-ter) and 87 (central)

TABLE IX

Test of immunoreactivity of the anti-SARS-CoV nucleoprotein Abs: optical densities measured with each combination of antibodies according to the dilutions of the inactivated viral antigen.

No.	Dilution	F/28	G/28	G/87	H/28	H/28 + 87
0	1/100	5	5	3.495	3.900	5
1	1/500	3.795	3.814	1.379	3.702	3.804
2	1/2 500	2.815	2.950	0.275	3.268	2.680
3	1/12 500	0.987	1.038	0.135	1.374	0.865
4	1/62 500	0.404	0.348	0.125	0.480	0.328
5	1/312 500	0.285	0.211	0.123	0.240	0.215
6	Control	0.210	0.200	0.098	0.186	0.156
7	Control	0.269	0.153	0.104	0.193	0.202

[0598] The detection limit for these 4 experimental trials corresponds to the antigen dilution in negative serum 1:62 500. A rapid extrapolation suggests the detection of less than 10^3 infectious particles per ml of sera.

[0599] From this study, it is evident that the most appropriate antibodies for the capture of the native viral nucleoprotein are the antibodies specific for the central region and/or for a conformational epitope, both being antibodies also selected for their high affinity for the native antigen.

[0600] Having determined the best antibodies for the composition of the solid phase, the antibodies to be selected as a priority for the detection of the antigens attached to the solid phase are the complementary antibodies specific for a dominant epitope in the C-ter region. The use of any other complementary antibody specific for epitopes located in the N-ter region of the protein leads to average or poor results.

EXAMPLE 11

Eukaryotic Expression Systems for the SARS-Associated Coronavirus (SARS-CoV) spicule (S) Protein

1) Optimization of the Conditions for Expression of the SARS-CoV S in Mammalian Cells

[0601] The conditions for transient expression of the SARS-CoV spicule (S) protein were optimized in mammalian cells (293T, VeroE6).

[0602] For that, a DNA fragment containing the cDNA for SARS-CoV S was amplified by PCR with the aid of the oligo-nucleotides 5'-ATAGGATCCA CCATGTTTAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-ATACTC-GAGTT ATGTGTAATG TAATTTGACA CCCTTG-3' from the plasmid pSARS-S(C.N.C.M. No. I-3059) and then inserted between the BamHI and Xho1 sites of the plasmid pTRIPΔU3-CMV containing a lentiviral vector TRIP (Sirven, 2001, Mol. Ther., 3, 438-448) in order to obtain the plasmid pTRIP-S. The BamH1 and Xho1 fragment containing the cDNA for S was then subcloned between BamH1 and Xho1 of the eukaryotic expression plasmid pcDNA3.1(+) (Clontech) in order to obtain the plasmid pcDNA-S. The Nhe1 and Xho1 fragment containing the cDNA for S was then subcloned between the corresponding sites of the expression plasmid pCI (Promega) in order to obtain the plasmid pCI-S. The WPRE sequences of the woodchuck hepatitis virus ("Woodchuck Hepatitis Virus posttranscriptional regulatory element") and the CTE sequences ("constitutive transport element") of the simian retro-virus from Mason-Pfizer were inserted into each of the two plasmids pcDNA-S and pCI-S between the Xho1 and Xba1 sites in order to obtain respectively the plasmids pcDNA-S-CTE, pcDNA-S-WPRE, pCI-S-CTE and pCI-S-WPRE (FIG. 21). The plasmid pCI-S-WPRE was deposited at the CNCM, on Nov. 22, 2004, under the number I-3323. All the inserts were sequenced with the aid of a BigDye Terminator v1.1 kit (Applied Biosystems) and an automated sequencer ABI377.

[0603] The capacity of the plasmid constructs to direct the expression of SARS-CoV S in mammalian cells was assessed after transfection of VeroE6 cells (FIG. 22). In this experiment, monolayers of 5×10^5 VeroE6 cells in 35 mm Petri dishes were transfected with 2 μg of plasmids pcDNA (as control), pcDNA-S, pCI and pCI-S and 6 μl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, cellular extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel, and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum from the rabbit P11135: cf. example 4 above) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and autoradiography films Hyperfilm MP (Amersham).

[0604] This experiment (FIG. 22) shows that the plasmid pcDNA-S does not make it possible to direct the expression of SARS-CoV S at detectable levels whereas the plasmid pCI-S allows a weak expression, close to the limit of detection, which may be detected when the film is overexposed. Similar results were obtained when the expression of S was sought by immunofluorescence (data not shown). This impossibility to detect effective expression of S cannot be attributed to the detection techniques used since the S protein can be detected at the expected size (180 kDa) in an extract of cells infected with SARS-CoV or in an extract of VeroE6 cells infected with the recombinant vaccinia virus VV-TF7.3 and transfected with the plasmid pcDNA-S. In this latter experiment, the virus VV-TF7.3 expresses the RNA polymerase of the T7 phage and allows the cytoplasmic transcription of an uncapped RNA capable of being efficiently translated. This experiment suggests that the expression defects described above are due to an intrinsic inability of the cDNA for S to be efficiently expressed when the step for transcription to messenger RNA is carried out at the nuclear level.

[0605] In a second experiment, the effect of the CTE and WPRE signals on the expression of S was assessed after transfection of VeroE6 (FIG. 23A) and 293T (FIG. 23B) cells and according to a protocol similar to that described above. Whereas the expression of S cannot be detected after transfection of the plasmids pcDNA-S-CTE and pcDNA-S-WPRE derived from pcDNA-S, the insertion of the WPRE and CTE signals greatly improves the expression of S in the context of the expression plasmid pCI-S.

[0606] To specify this result, a second series of experiments were carried out where the immunoblot is quantitatively visualized by luminescence and acquisition on a

digital imaging device (Fluor S, BioRad). The analysis of the results obtained with the QuantityOne v4.2.3 software (Bio-Rad) shows that the WPRE and CTE sequences increase respectively the expression of S by a factor of 20 to 42 and 10 to 26 in Vero E6 cells (table X). In 293T cells (table X), the effect of the CTE sequence is more moderate (4 to 5 times) whereas that of the WPRE sequence remains high (13 to 28 times).

TABLE X

Quantitative analysis of the effect of the CTE and WPRE signals on the expression of SARS-CoV S: Cellular extracts were prepared 48 hours after transfection of VeroE6 or 293T cells with the plasmid pCI, pCI-S, pCI-S-CTE and pCI-S-WPRE and analyzed by Western blotting as described in the legend to FIG. 22. The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (FluorS, BioRad). The expression levels are indicated according to an arbitrary scale where the value of 1 represents the level measured after transfection of the plasmid pCI-S. Two independent experiments were carried out for each of the two cell types. In experiment 1 on VeroE6 cells, the transfections were carried out in duplicate and the results are indicated in the form of the mean and standard deviation values for the expression levels measured.

Plasmid	cell	exp. 1	exp. 2
PCI	VeroE6	0.0	0.0
pCI-S	VeroE6	1.0 ± 0.1	1.0
pCI-S-CTE	VeroE6	9.8 ± 0.9	26.4
pCI-S-WPRE	VeroE6	20.1 ± 2.0	42.3
PCI	293T	0.0	0.0
PCI-S	293T	1.0	1.0
PCI-S-CTE	293T	4.6	4.0
PCI-S-WPRE	293T	27.6	12.8

[0607] In summary, all these results show that the expression, in mammalian cells, of the cDNA for the SARS-CoV S under the control of the RNA polymerase II promoter sequences requires, to be efficient, the expression of a splice signal and of either of the sequences WPRE and CTE.

2) Production of Stable Lines Allowing the Expression of SARS-CoV S

[0608] The cDNA for the SARS-CoV S protein was cloned in the form of a BamH1-Xho1 fragment into the plasmid pTRIPΔU3-CMV containing a defective lentiviral vector TRIP with central DNA flap (Sirven et al., 2001, Mol. Ther., 3: 438-448) in order to obtain the plasmid pTRIP-S (FIG. **24**). Transient cotransfection according to Zennou et al. (2000, Cell, 101: 173-185) of this plasmid, of an encapsidation plasmid (p8.2) and of a plasmid for expression of the VSV envelope glycoprotein G (pHCMV-G) in 293T cells allowed the preparation of retroviral pseudoparticles containing the vector TRIP-S and pseudotyped with the envelope protein G. These pseudotyped TRIP-S vectors were used to translate 293T and FRhK-4 cells: no expression of the S protein could be detected by Western blotting and immunofluorescence in the transduced cells (data not presented).

[0609] The optimum expression cassettes consisting of the CMV virus immediate/early promoter, a splice signal, cDNA for S and either of the posttranscriptional signals WPRE or CTE described above were then substituted for the EF1α-EGFP cassette of the defective lentiviral expression vector with central DNA flap TRIPΔU3-EF1α (Sirven et al., 2001, Mol. Ther., 3: 438-448) (FIG. **25**). These substitutions were carried out by a series of successive subclonings of the S expression cassettes which were excised from the plasmids pCT-S-CTE (BglII-Apa1) or respectively pCI-S-WPRE (BglII-Sal1) and then inserted between the Mlu1 and Kpn1 sites or respectively Mlu1 or Xho1 sites of the plasmid TRIPΔU3-EF1α in order to obtain the plasmids pTRIP-SD/SA-S-CTE and pTRIP-SD/SA-S-WPRE, deposited at the CNCM, on Dec. 1, 2004, under the numbers I-3336 and I-3334, respectively. Pseudotyped vectors were produced according to Zennou et al. (2000, Cell, 101: 173-185) and used to transduce 293T cells (10 000 cells) and FRhK-4 cells (15 000 cells) according to a series of 5 successive transduction cycles with a quantity of vectors corresponding to 25 ng (TRIP-SD/SA-S-CTE) or 22 ng TRIP-SD/SA-S-WPRE) of p24 per cycle.

[0610] The transduced cells were cloned by limiting dilution and a series of clones were qualitatively analyzed for the expression of SARS-CoV S by immunofluorescence (data not shown), and then quantitatively by Western blotting (FIG. **25**) with the aid of an anti-S rabbit polyclonal serum. The results presented in FIG. **25** show that clones 2 and 15 of FrhK4-s-CTE cells transduced with TRIP-SD/SA-S-CTE and clones 4, 9 and 12 of FRhK4-S-WPRE cells transduced with TRIP-SD/SA-S-WPRE allow the expression of the SARS-CoV S at respectively low or moderate levels if they are compared to those which can be observed during infection with SARS-CoV.

[0611] In summary, the vectors TRIP-SD/SA-S-CTE and TRIP-SD/SA-S-WPRE allow the production of stable clones of FRhK-4 cells and similarly 293T cells expressing SARS-CoV S, whereas the assays carried out with the "parent" vector TRIP-S remained unsuccessful, which demonstrates the need for a splice signal and for either of the sequences CTE and WPRE for the production of stable cell clones expressing the S protein.

[0612] In addition, these modifications of the vector TRIP (insertion of a splice signal and of a post-transcriptional signal like CTE and WPRE) could prove advantageous for improving the expression of other cDNAs than that for S.

[0613] 3) Production of stable lines allowing the expression of a soluble form of SARS-CoV S. Purification of this recombinant antigen.

[0614] A cDNA encoding a soluble form of the S protein (Ssol) was obtained by fusing the sequences encoding the ecto-domain of the protein (amino acids 1 to 1193) with those of a tag (FLAG:DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. Practically, in order to obtain the plasmid pcDNA-Ssol, a DNA fragment encoding the ectodomain of SARS-CoV S was amplified by PCR with the aid of the oligonucleotides 5'-ATAGGATCCA CCATGTT-TAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-AC-CTCCGGAT TTAATATATT GCTCATATTT TCCCAA-3' from the plasmid pcDNA-S, and then inserted between the unique BamH1 and BspE1 sites of a modified eukaryotic expression plasmid pcDNA3.1(+) (Clontech) containing the tag sequence FLAG between its BamH1 and Xho1 sites:

```
// GGATCC ...nnn... TCC GGA GAT TAT AAA GAT GAC
    BamH1            S   G   D   Y   K   D   D

GAC GAT AAA TAA CTCGAG //
 D   D   K  ter Xhol
```

[0615] The Nhe1-Xho1 and BamH1-Xho1 fragments, containing the cDNA for S, were then excised from the plasmid pcDNA-Ssol, and subcloned between the corresponding sites of the plasmid pTRIP-SD/SA-S-CTE and of the plasmid pTRIP-SD-SA-S-WPRE, respectively, in order to obtain the plasmids pTRIP-SD/SA-Ssol-CTE and pTRIP-SD/SA-Ssol-WPRE, deposited at the CNCM, on Dec. 1, 2004, under the numbers I-3337 and I-3335, respectively.

[0616] Pseudotyped vectors were produced according to Zennou et al. (2000, Cell, 101:173-185) and used to transduce FRhK-4 cells (15 000 cells) according to a series of 5 successive transduction cycles (15 000 cells) with a quantity of vector corresponding to 24 ng (TRIP-SD/SA-Ssol-CTE) or 40 ng (TRIP-SD/SA-Ssol-WPRE) of p24 per cycle. The transduced cells were cloned by limiting dilution and a series of 16 clones transduced with TRIP-SD/SA-Ssol-CTE and of 15 clones with TRIP-SD/SA-Ssol-WPRE were analyzed for the expression of the Ssol polypeptide by Western blotting visualized with an anti-FLAG monoclonal antibody (FIG. **26** and data not presented), and by capture ELISA specific for the Ssol polypeptide which was developed for this purpose (table XI and data not presented). Part of the process for selecting the best secretory clones is shown in FIG. **26**. Capture ELISA is based on the use of solid phases coated with polyclonal antibodies of rabbits immunized with purified and inactivated SARS-CoV. These solid phases allow the capture of the Ssol polypeptide secreted into the cellular supernatants, whose presence is then visualized with a series of steps successively involving the attachment of an anti-FLAG monoclonal antibody (M2, SIGMA), of anti-mouse IgG(H+L) biotinylated rabbit polyclonal antibodies (Jackson) and of a streptavidin-peroxidase conjugate (Amersham) and then the addition of chromogen and substrate (TMB+H_2O_2, KPL).

TABLE XI

Analysis of the expression of the Ssol polypeptide by cell lines transduced with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE. The secretion of the Ssol polypeptide was assessed in the supernatant of a series of cell clones isolated after transduction of FRhK-4 cells with the lentiviral vectors TRIP-SD/SA-Ssol-WPRE and TRIP-SD/SA-Ssol-CTE. The supernatants diluted 1/50 were analyzed by a capture ELISA test specific for SARS-CoV S.

Vector	Clone	OD (450 nm)
Control	—	0.031
TRIP-SD/SA-Ssol-CTE	CTE2	0.547
	CTE3	0.668
	CTE9	0.171
	CTE12	0.208
	CTE13	0.133
TRIP-SD/SA-Ssol-WPRE	WPRE1	0.061
	WPRE10	0.134

[0617] The cell line secreting the highest quantities of Ssol polypeptide in the culture supernatant is the FRhK4-Ssol-CTE3 line. It was subjected to a second series of 5 cycles of transduction with the vector TRIP-SD/SA-Ssol-CTE under conditions similar to those described above and then cloned. The subclone secreting the highest quantities of Ssol was selected by a combination of Western blot and capture ELISA analysis: it is the subclone FRhK4-Ssol-30, which was deposited at the CNCM, on Nov. 22, 2004, under the name I-3325.

[0618] The FRhK4-Ssol-30 line allows the quantitative production and purification of the recombinant Ssol polypeptide. In a typical experiment where the experimental conditions for growth, production and purification were optimized, the cells of the FRhK4-Ssol-30 line are inoculated in standard culture medium (pyruvate-free DMEM containing 4.5 g/l of glucose and supplemented with 5% FCS, 100 U/ml of penicillin and 100 μg/ml of streptomycin) in the form of a subconfluent monolayer (1 million cells per each 100 cm^2 in 20 ml of medium). At confluence, the standard medium is replaced with the secretion medium where the quantity of FCS is reduced to 0.5% and the quantity of medium reduced to 16 ml per each 100 cm^2. The culture supernatant is removed after 4 to 5 days of incubation at 35° C. and under 5% CO_2. The recombinant polypeptide Ssol is purified from the supernatant by the succession of steps of filtration on 0.1 μm polyethersulfone (PES) membrane, concentration by ultrafiltration on a PES membrane with a 50 kD cut-off, affinity chromatography on anti-FLAG matrix with elution with a solution of FLAG peptide (DYKDDDDK) at 100 μg/ml in TBS (50 mM tris, pH 7.4, 150 mM NaCl) and then gel filtration chromatography in TBS on sephadex G-75 beads (Pharmacia). The concentration of the purified recombinant Ssol polypeptide was determined by micro-BCA test (Pierce) and then its biochemical characteristics analyzed.

[0619] Analysis by 8% SDS acrylamide gel stained with silver nitrate demonstrates a predominant polypeptide whose molecular mass is about 180 kD and whose degree of purity may be evaluated at 98% (FIG. **27**A). Two main peaks are detected by SELDI-TOF mass spectrometry (Cyphergen): they correspond to single and double charged forms of a predominant polypeptide whose molecular mass is thus determined at 182.6±3.7 kD (FIGS. **27**B and C). After transfer onto Prosorb membrane and rinsing in 0.1% TFA, the N-terminal end of the Ssol polypeptide was sequenced in liquid phase by Edman degradation on 5 residues (ABI494, Applied Biosystems) and determined as being SDLDR (FIG. **27**D). This demonstrates that the signal peptide located at the N-terminal end of the SARS-CoV S protein, composed of aa 1 to 13 (MFIFLLFLTLTSG) according to an analysis carried out with the software signalP v2.0 (Nielsen et al., 1997, *Protein Engineering*, 10:1-6), is cleaved from the mature Ssol polypeptide. The recombinant Ssol polypeptide therefore consists of amino acids 14 to 1193 of the SARS-CoV S protein fused at the C-terminals with a sequence SGDYKDDDDK containing the sequence of the FLAG tag (underlined). The difference between the theoretical molar mass of the naked Ssol polypeptide (132.0 kD) and the real molar mass of the mature polypeptide (182.6 kD) suggests that the Ssol polypeptide is glycosylated.

[0620] A preparation of purified Ssol polypeptide, whose protein concentration was determined by micro-BCA test, makes it possible to prepare a calibration series in order to measure, with the aid of the capture ELISA test described

above, the concentrations of Ssol present in the culture supernatants and to review the characteristics of the secretory lines. According to this test, the FRhK4-Ssol-CT3 line secretes 4 to 6 g/ml of polypeptide Ssol while the FRhK4-Ssol-30 line secretes 9 to 13 g/ml of Ssol after 4 to 5 days of culture at confluence. In addition, the purification scheme presented above makes it possible routinely to purify from 1 to 2 mg of Ssol polypeptide per liter of culture supernatant.

EXAMPLE 12

Gene Immunization Involving the SARS-Associated Corona Virus (SARS-CoV) Spicule (S) Protein

[0621] The effect of a splice signal and of the posttranscriptional signals WPRE and CTE was analyzed after gene immunization of BALB/c mice (FIG. **28**).

[0622] For that, BALB/c mice were immunized at intervals of 4 weeks by injecting into the tibialis anterior a saline solution of 50 μg of plasmid DNA of pcDNA-S and pCI-S and, as a control, 50 μg of plasmid DNA of pcDNA-N (directing the expression of SARS-CoV N) or of pCI-HA (directing the expression of the HA of the influenza virus A/PR/8/34) and the immune sera collected 3 weeks after the 2nd injection. The presence of antibodies directed against the SARS-CoV S was assessed by indirect ELISA using as antigen a lysate of VeroE6 cells infected with SARS-CoV and, as a control, a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers (TI) are calculated as the reciprocal of the dilution producing a specific OD of 0.5 (difference between OD measured on a lysate of infected cells and OD measured on a lysate of noninfected cells) after visualization with an anti-mouse IgG polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL) (FIG. **28**A).

[0623] Under these conditions, the expression plasmid pcDNA-S only allows the induction of low antibody titers directed against SARS-CoV S in 3 mice out of 6 (LOG_{10}(TI)=1.9±0.6) whereas the plasmid pcDNA-N allows the induction of anti-N antibodies at high titers (LOG_{10}(TI)=3.9±0.3) in all the animals, and the control plasmids (pCI, pCI-HA) do not result in any detectable antibody (LOG_{10}(TI)<1.7). The plasmid pCI-S equipped with a splice signal allows the induction of antibodies at high titers (LOG_{10}(TI)=3.7±0.2), which are approximately 60 times higher than those observed after injection of the plasmid pcDNA-S ($p<10^{-5}$).

[0624] The efficiency of the posttranscriptional signals was studied by carrying out a dose-response study of the anti-S antibody titers induced in the BALB/c mouse as a function of the quantity of plasmid DNA used as immunogen (2 μg, 10 μg and 50 μg). This study (FIG. **28**B) demonstrates that the posttranscriptional signal WPRE greatly improves the efficiency of gene immunization when small doses of DNA are used ($p<10^{-5}$ for a dose of 2 μg of DNA and $p<10^{-2}$ for a dose of 10 μg), whereas the effect of the CTE signal remains marginal (p=0.34 for a dose of 2 μg of DNA).

[0625] Finally, the antibodies induced in mice after gene immunization neutralize the infectivity of SARS-CoV in vitro (FIGS. **29**A and **29**B) at titers which are consistent with the titers measured by ELISA.

[0626] In summary, the use of a splice signal and of the posttranscriptional signal WPRE of the woodchuck hepatitis virus considerably improves the induction of neutralizing antibodies directed against SARS-CoV after gene immunization with the aid of plasmid DNA directing the expression of the cDNA for SARS-CoV S.

EXAMPLE 13

Diagnostic Applications of the S Protein

[0627] The ELISA reactivity of the recombinant Ssol polypeptide was analyzed with respect to sera from patients suffering from SARS.

[0628] The sera from probable cases of SARS tested were chosen on the basis of the results (positive or negative) of analysis of their specific reactivity toward the native antigens of SARS-CoV by immunofluorescence test on VeroE6 cells infected with SARS-CoV and/or by indirect ELISA test using as antigen a lysate of VeroE6 cells infected with SARS-CoV. The sera of these patients are identified by a serial number of the National Reference Center for Influenza Viruses and by the initials of the patient and the number of days elapsed since the onset of the symptoms. All the sera of probable cases (cf. Table XII) recognize the native antigens of SARS-CoV, with the exception of the serum 032552 of the patient VTT for whom infection with SARS-CoV could not be confirmed by RT-PCR performed on respiratory samples of days 3, 8 and 12. A panel of control sera was used as control (TV sera): they are sera collected in France before the SARS epidemic that occurred in 2003.

TABLE XII

Sera of probable cases of SARS

Serum	Patient	Sample collection day
031724	JYK	7
033168	JYK	38
033597	JYK	74
032632	NTM	17
032634	THA	15
032541	PHV	10
032542	NIH	17
032552	VTT	8
032633	PTU	16
032791	JLB	3
033258	JLB	27
032703	JCM	8
033153	JCM	29

[0629] Solid phases sensitized with the recombinant Ssol polypeptide were prepared by adsorption of a solution of purified Ssol polypeptide at 2 μg/ml in PBS in the wells of an ELISA plate, and then the plates are incubated overnight at 4° C. and washed with PBS-Tween buffer (PBS, 0.1% Tween 20). After saturating the ELISA plates with a solution of PBS-10% skimmed milk (weight/volume) and washing in PBS-Tween, the sera to be tested (100 μl) are diluted 1/400 in PBS skimmed milk-Tween buffer (PBS, 3% skimmed milk, 0.1% Tween) and then added to the wells of the sensitized ELISA plate. The plates are incubated for 1 h at 37° C. After 3 washings with PBS-Tween buffer, the anti-human IgG conjugate labeled with peroxidase (ref. NA933V, Amersham) diluted 1/4000 in PBS-skimmed milk-Tween buffer is

added, and then the plates are incubated for 1 hour at 37° C. After 6 washings with PBS-Tween buffer, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 10 minutes protected from light. The reaction is stopped by adding a 1 N H_3PO_4 solution, and then the absorbance is measured at 450 nm with a reference at 620 nm.

[0630] The ELISA tests (FIG. 30) demonstrate that the recombinant Ssol polypeptide is specifically recognized by the serum antibodies of patients suffering from SARS collected at the medium or late phase of infection (≧10 days after the onset of the symptoms) whereas it is not significantly recognized by the serum antibodies of 2 patients (JLB and JCM) collected in the early phase of infection (3 to 8 days after the onset of the symptoms) or by control sera of subjects not suffering from SARS. The serum antibodies of patients JLB and JCM show a seroconversion between days 3 and 27 for the first and 8 and 29 for the second after the onset of the symptoms, which confirms the specificity of the reactivity of these sera toward the Ssol polypeptide.

[0631] In conclusion, these results demonstrate that the recombinant Ssol polypeptide may be used as an antigen for the development of an ELISA test for serological diagnosis of infection with SARS-CoV.

EXAMPLE 14

Vaccine Applications of the Recombinant Soluble S Protein

[0632] The immunogenicity of the recombinant Ssol polypeptide was studied in mice.

[0633] For that, a group of 6 mice was immunized at 3 weeks' interval with 10 μg of recombinant Ssol polypeptide adjuvanted with 1 mg of aluminum hydroxide (Alu-gel-S, Serva) diluted in PBS. Three successive immunizations were performed and the immune sera were collected 3 weeks after each of the immunizations (IS1, IS2, IS3). As a control, a group of mice (mock group) received aluminum hydroxide alone according to the same protocol.

[0634] The immune sera were analyzed per pool for each of the 2 groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen and as a control a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG(H+L) polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL). This analysis (FIG. 31) shows that the immunization with the Ssol polypeptide induces in mice, from the first immunization, antibodies directed against the native form of the SARS-CoV spicule protein present in the lysate of infected VeroE6 cells. After 2 then 3 immunizations, the anti-S antibody titers become very high.

[0635] The immune sera were analyzed per pool for each of the two groups for their capacity to seroneutralize the infectivity of SARS-CoV. 4 points of seroneutralization on FRhK-4 cells (100 TCID50 of SARS-CoV) are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4. This analysis shows that the antibodies induced in mice by the Ssol polypeptide are neutralizing: the titers observed are very high after 2 and then 3 immunizations (greater than 2560 and 5120 respectively, table XIII).

TABLE XIII

Induction of antibodies directed against SARS-CoV after immunization with the recombinant Ssol polypeptide. The immune sera were analyzed per pool for each of the two groups for their capacity to seroneutralize the infectivity of 100 TCID50 of SARS-CoV on FRhK-4 cells. 4 points are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4.

Group	Sera	Neutralizing Ab
Mock	pi	<20
	IS1	<20
	IS2	<20
	IS3	<20
Ssol	pi	<20
	IS1	57
	IS2	>2560
	IS3	>5120

[0636] The neutralizing titers observed in mice immunized with the Ssol polypeptide reach levels far greater than the titers observed by Yang et al. in mice (2004, Nature, 428:561-564) and those observed by Buchholz in the hamster (2004, PNAS 101:9804-9809) which protect respectively mice and hamsters from infection with SARS-CoV. It is therefore probable that the neutralizing antibodies induced in mice after immunization with the Ssol polypeptide protect these animals against infection with SARS-CoV.

EXAMPLE 15

Optimized Synthetic Gene for the Expression in Mammalian Cells of the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein

1) Design of the Synthetic Gene

[0637] A synthetic gene encoding the SARS-CoV spicule protein was designed from the gene of the isolate 031589 (plasmid pSARS-S, C.N.C.M. No. I-3059) so as to allow high levels of expression in mammalian cells and in particular in cells of human origin.

[0638] For that:

[0639] the use of codons of the wild-type gene of the isolate 031589 was modified so as to become close to the bias observed in humans and to improve the efficiency of translation of the corresponding mRNA

[0640] the overall GC content of the gene was increased so as to extend the half-life of the corresponding mRNA

[0641] the optionally cryptic motifs capable of interfering with an efficient expression of the gene were deleted (splice donor and acceptor sites, polyadenylation signals, sequences very rich (>80%) or very low (<30%) in GC, repeat sequences, sequences involved in the formation of secondary RNA structures, TATA boxes)

[0642] a second STOP codon was added to allow efficient termination of translation.

[0643] In addition, CpG motifs were introduced into the gene so as to increase its immunogenicity as DNA vaccine. In order to facilitate the manipulation of the synthetic gene, two BamH1 and Xho1 restriction sites were placed on either side of the open reading frame of the S protein, and the BamH1, Xho1, Nhe1, Kpn1, BspE1 and Sal1 restriction sites were avoided in the synthetic gene.

[0644] The sequence of the synthetic gene designed (gene 040530) is given in SEQ ID No: 140.

[0645] An alignment of the synthetic gene 040530 with the sequence of the wild-type gene of the isolate 031589 of SARS-CoV deposited at the C.N.C.M. under the number I-3059 (SEQ ID No: 4, plasmid pSRAS-S) is presented in FIG. **32**.

2) Plasmid Constructs

[0646] The synthetic gene SEQ ID No: 140 was assembled from synthetic oligonucleotides and cloned between the Kpn1 and Sac1 sites of the plasmid pUC-Kana in order to give the plasmid 040530pUC-Kana. The nucleotide sequence of the insert of the plasmid 040530pUC-Kana was verified by automated sequencing (Applied).

[0647] A Kpn1-Xho1 fragment containing the synthetic gene 040530 was excised from the plasmid 040530pUC-Kana and subcloned between the Nhe1 and Xho1 sites of the expression plasmic pCI (Promega) in order to obtain the plasmid pCI-SSYNTH, deposited at the CNCM on Dec. 1, 2004, under the number I-3333.

[0648] A synthetic gene encoding the soluble form of the S protein was then obtained by fusing the synthetic sequences encoding the ectodomain of the S protein (amino acids 1 to 1193) with those of the tag (FLAG:DYKDDDDK) via a linker BspE1 encoding the dipeptide SG. Practically, a DNA fragment encoding the ectodomain of the SARS-CoV S was amplified by PCR with the aid of the oligonucleotides 5'-ACTAGCTAGCGGATCCACCATGTTCATCTT CCTG-3' and 5'-AGTATCCGGAC TTG ATGTACT GCTCG-TACTTGC-3' from the plasmid 040530pUC-Kana, digested with Nhe1 and BspE1 and then inserted between the unique Nhe1 and BspE1 sites of the plasmid pCI-Ssol, to give the plasmid pCI-SCUBE, deposited at the CNCM on Dec. 1, 2004, under the number I-3332. The plasmids pCI-Ssol, pCI-Ssol-CTE, and pCI-Ssol-WPRE (deposited at the CNCM, on Nov. 22, 2004, under the number I-3324) had been previously obtained by subcloning the Kpn1-Xho1 fragment excised from the plasmid pcDNA-Ssol (see technical note of DI 2004-106) between the Nhe1 and Xho1 sites of the plasmids pCI, pCI-S-CTE and pCI-S-WPRE respectively.)

[0649] The plasmids pCI-Scube and pCI-Ssol encode the same recombinant Ssol polypeptide.

3) Results

[0650] The capacity of the synthetic gene encoding the S protein to efficiently direct the expression of the SARS-CoV S in mammalian cells was compared with that of the wild-type gene after transient transfection of primate cells (VeroE6) and of human cells (293T).

[0651] In the experiment presented in FIG. **33** and in table XIV, monolayers of 5×10^5 VeroE6 cells or 7×10^5 293T cells in 35 mm Petri dishes were transfected with 2 g of plasmids pCI (as control), pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-S-Ssynth and 6 µl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, cell extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum of the rabbit P11135: cf example 4 above) and of donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The immunoblot was quantitatively visualized by luminescence with the aid of the ECL+ kit (Amersham) and acquisition on a digital imaging device (Fluor S, BioRad).

[0652] The analysis of the results obtained with the software QuantityOne v4.2.3 (BioRad) shows that in this experiment, the plasmid pCI-Synth allows the transient expression of the S protein at high levels in the VeroE6 and 293T cells, whereas the plasmid pCI-S does not make it possible to induce expression at sufficient levels to be detected. The expression levels observed are of the order of twice as high as those observed with the plasmid pCI-S-WPRE.

TABLE XIV

Use of a synthetic gene for the expression of the SARS-CoV S. Cell extracts prepared 48 hours after transfection of VeroE6 or 293T cells with the plasmids pCI, pCI-S, pCI-S-CTE, pCI-S-WPRE and pCI-S-Ssynth were separated on 8% SDS acrylamide gel and analyzed by Western blotting with the aid of an anti-S rabbit polyclonal antibody and an anti-rabbit IgG (H + L) polyclonal antibody coupled with peroxidase (NA934V, Amersham). The Western blot is visualized by luminescence (ECL+, Amersham) and acquisition on a digital imaging device (FluorS, BioRad). The expression levels of the S protein were measured by quantifying the two predominant bands identified on the image (see FIG. 33) and are indicated according to an arbitrary scale where the value 1 represents the level measured after transfection of the plasmid pCI-S-WPRE.

Plasmid	VeroE6	293T
pCI	0.0	0.0
pCI-S	≦0.1	≦0.1
pCI-S-CTE	0.5	≦0.1
pCI-S-WPRE	1.0	1.0
pCI-Ssynth	1.8	1.9

[0653] In a second instance, the capacity of the synthetic gene Scube to efficiently direct the synthesis and the secretion of the Ssol polypeptide by mammalian cells was compared with that of the wild-type gene after transient transfection of hamster cells (BHK-21) and of human cells (293T).

[0654] In the experiment presented in table XV, monolayers of 6×10^5 BHK-21 cells and 7×10^5 293T cells in 35 mm Petri dishes were transfected with 2 µg of plasmids pCI (as control), pCI-Ssol, pCI-Ssol-CTE, pCI-Ssol-WPRE and pCI-Scube and 6 µl of Fugene6 reagent according to the manufacturer's instructions (Roche). After 48 hours of incubation at 37° C. and under 5% CO_2, the cellular supernatants

were collected and quantitatively analyzed for the secretion of the Ssol polypeptide by a capture ELISA test specific for the Ssol polypeptide.

[0655] Analysis of the results shows that, in this experiment, the plasmid pCI-Scube allows the expression of the Ssol polypeptide at levels 8 times (BHK-21 cells) to 20 times (293T cells) higher than the plasmid pCI-Ssol.

[0656] The levels of expression observed are of the order of twice (293T cells) to 5 times (BHK-21 cells) as high as those observed with the plasmid pCI-Ssol-WPRE.

TABLE XV

Use of a synthetic gene for the expression of the Ssol polypeptide. The supernatants were harvested 48 hours after transfection of BHK or 293T cells with the plasmids pCI, pCI-Ssol, pCI-Ssol-CTE, pCI-Ssol-WPRE and pCI-Scube and quantitatively analyzed for the secretion of the Ssol polypeptide by an ELISA test specific for the Ssol polypeptide. The transfections were carried out in duplicate and the results are presented in the form of means and standard deviations of the concentrations of Ssol polypeptide (ng/ml) measured in the supernatants.

Plasmid	BHK	293T
pci	<20	<20
pCI-Ssol	<20	56 ± 10
pCI-Ssol-CTE	<20	63 ± 8
pCI-Ssol-WPRE	28 ± 1	531 ± 15
pCI-Scube	152 ± 6	1140 ± 20

[0657] In summary, these results show that the expression, in mammalian cells, of the synthetic gene 040530 encoding SARS-CoV S under the control of RNA polymerase II promoter sequences is much more efficient than that of the wild-type gene of the 031589 isolate. This expression is even more efficient than that directed by the wild-type gene in the presence of the WPRE sequences of the woodchuck hepatitis virus.

4) Applications

[0658] The use of the synthetic gene 040530 encoding SARS-CoV S or its Scube variant encoding the polypeptide Ssol is capable of advantageously replacing the wild-type gene in numerous applications where the expression of S is necessary at high levels. In particular in order to:

[0659] improve the efficiency of gene immunization with plasmids of the pCI-Ssynth or even pCI-Ssynth-CTE or pCI-Ssynth-WPRE type

[0660] establish novel cell lines expressing higher quantities of the S protein or of the Ssol polypeptide with the aid of recombinant lentiviral vectors carrying the Ssynth gene or the Scube gene respectively

[0661] improve the immunogenicity of the recombinant lentiviral vectors allowing the expression of the S protein or of the Ssol polypeptide

[0662] improve the immunogenicity of live vectors allowing the expression of the S protein or of the Ssol polypeptide like recombinant vaccinia viruses or recombinant measles viruses (see examples 16 and 17 below)

EXAMPLE 16

Expression of the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein with the Aid of Recombinant Vaccinia Viruses

Vaccine Application

Application to the Production of a Soluble form of the Spicule (S) Protein and Design of a Serological Test for SARS

1) Introduction

[0663] The aim of this example is to evaluate the capacity of recombinant vaccinia viruses (VV) expressing various SARS-associated coronavirus (SARS-CoV) antigens to constitute novel vaccine candidates against SARS and a means of producing recombinant antigens in mammalian cells.

[0664] For that, the inventors focused on the SARS-CoV spicule (S) protein which makes it possible to induce, after gene immunization in animals, antibodies neutralizing the infectivity of SARS-CoV, and a soluble and secreted form of this protein, the Ssol polypeptide, which is composed of the ectodomain (aa 1-1193) of S fused at its C-ter end with a tag FLAG (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. This Ssol polypeptide exhibits an antigenicity similar to that of the S protein and allows, after injection into mice in the form of a purified protein adjuvanted with aluminum hydroxide, the induction of high neutralizing antibody titers against SARS-CoV.

[0665] The various forms of the S gene were placed under the control of the promoter of the 7.5K gene and then introduced into the thymidine kinase (TK) locus of the Copenhagen strain of the vaccinia virus by double homologous recombination in vivo. In order to improve the immunogenicity of the recombinant vaccinia viruses, a synthetic late promoter was chosen in place of the 7.5K promoter, in order to increase the production of S and Ssol during the late phases of the viral cycle.

[0666] After having isolated the recombinant vaccinia viruses and verified their capacity to express the SARS-CoV S antigen, their capacity to induce in mice an immune response against SARS was tested. After having purified the Ssol antigen from the supernatant of infected cells, an ELISA test for serodiagnosis of SARS was designed, and its efficiency was evaluated with the aid of sera from probable cases of SARS.

2) Construction of the Recombinant Viruses

[0667] Recombinant vaccinia viruses directing the expression of the S glycoprotein of the 031589 isolate of SARS-CoV and of a soluble and secreted form of this protein, the Ssol polypeptide, under the control of the 7.5K promoter were obtained. With the aim of increasing the levels of expression of S and Ssol, recombinant viruses in which the cDNAs for S and for Ssol are placed under the control of a late synthetic promoter were also obtained.

[0668] The plasmid pTG186poly is a transfer plasmid for the construction of recombinant vaccinia viruses (Kieny, 1986, Biotechnology, 4:790-795). As such, it contains the VV thymidine kinase gene into which the promoter of the 7.5K gene has been inserted followed by a multiple cloning site allowing the insertion of heterologous genes (FIG. 34A).

The promoter of the 7.5K gene in fact contains a tandem of two promoter sequences that are respectively active during the early (P_E) and late (P_L) phases of the vaccinia virus replication cycle. The BamH1-Xho1 fragments were excised from the plasmids pTRIP-S and pcDNA-SsoI respectively and inserted between the BamH1 and Sma1 sites of the plasmid pTG186poly in order to give the plasmids pTG-S and pTG-SsoI (FIG. **34**A). The plasmids pTG-S and pTG-SsoI were deposited at the CNCM, on Dec. 2, 2004, under the numbers I-3338 and I-3339, respectively.

[0669] The plasmids pTN480, pTN-S and pTN-SsoI were obtained from the plasmids pTG186poly, pTG-S and pTG-SsoI respectively, by substituting the Nde1-Pst1 fragment containing the 7.5K promoter by a DNA fragment containing the synthetic late promoter 480, which was obtained by hybridization of the oligonucleotides 5'-TATGAGCTTT TTTTTTTTTT TTTTTTTGGC ATATAAATAG ACTCG-GCGCG CCATCTGCA-3' and 5'-GATGGCGCGC-CGAGTCTATT TATATGCCAA AAAAAAAAAA AAAAAAAAGC TCA-3' (FIG. **34**B). The insert was sequenced with the aid of a BigDye Terminator v1.1 kit (Applied Biosystems) and an automated sequencer ABI377. The sequence of the late synthetic promoter 480 as cloned into the transfer plasmids of the pTN series is indicated in FIG. **34**C. The plasmids pTN-S and pTN-SsoI were deposited at the CNCM, on Dec. 2, 2004, under the numbers I-3340 and I-3341, respectively.

[0670] The recombinant vaccinia viruses were obtained by double homologous recombination in vivo between the TK cassette of the transfer plasmids of the series pTG and pTN and the TK gene of the Copenhagen strain of the vaccinia virus according to a procedure described by Kieny et al. (1984, Nature, 312:163-166). Briefly, CV-1 cells are transfected with the aid of DOTAP (Roche) with genomic DNA of the Copenhagen strain of the vaccinia virus and each of the transfer plasmids of the pTG and pTN series described above, and then superinfected with the helper vaccinia virus W-ts7 for 24 hours at 33° C. The helper virus is counter-selected by incubation at 40° C. for 2 days and then the recombinant viruses (TK-phenotype) selected by two cloning cycles under agar medium on 143Btk-cells in the presence of BuDr (25 μg/ml). The 6 viruses VV-TG, VV-TG-S, VV-TG-SsoI, VV-TN, VV-TN-S, and VV-TN-SsoI are respectively obtained with the aid of the transfer plasmids pTG186poly, pTG-S, pTG-SsoI, pTN480, pTN-S, pTN-SsoI. The viruses VV-TG and VV-TN do not express any heterologous gene and were used as TK-control in the experiments. The preparations of recombinant viruses were performed on monolayers of CV-1 or BHK-21 cells and the titer in plaque forming units (p.f.u) determined on CV-1 cells according to Earl and Moss (1998, Current Protocols in Molecular Biology, 16.16.1-16.16.13).

3) Characterization of the Recombinant Viruses

[0671] The expression of the transgenes encoding the S protein and the SsoI polypeptide was assessed by Western blotting.

[0672] Monolayers of CV-1 cells were infected at a multiplicity of 2 with various recombinant vaccinia viruses VV-TG, VV-TG-S, VV-TG-SsoI, W-TN, W-TN-S and VV-TN-SsoI. After 18 hours of incubation at 37° C. and under 5% CO2, cellular extracts were prepared in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and then transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was performed with the aid of an anti-S rabbit polyclonal serum (immune serum from the rabbit P11135: cf. example 4) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and autoradiography films Hyperfilm MP (Amersham).

[0673] As shown in FIG. **35**A, the recombinant virus VV-TN-S directs the expression of the S protein at levels which are comparable to those which can be observed 8 h after infection with SARS-CoV but which are much higher than those which can be observed after infection with VV-TG-S. In a second experiment (FIG. **35**B), the analysis of variable quantities of cellular extracts shows that the levels of expression observed after infection with viruses of the TN series (VV-TN-S and VV-TN-SsoI) are about 10 times as high as those observed with the viruses of the TG series (VV-TG-S and VV-TG-SsoI, respectively). In addition, the SsoI polypeptide is secreted into the supernatant of CV-1 cells infected with the VV-TN-SsoI virus more efficiently than in the supernatant of cells infected with VV-TG-SsoI (FIG. **36**A). In this experiment, the VV-TN-Sflag virus was used as a control because it expresses the membrane form of the S protein fused at its C-ter end with the FLAG tag. The Sflag protein is not detected in the supernatant of cells infected with VV-TN-Sflag, demonstrating that the SsoI polypeptide is indeed actively secreted after infection with VV-TN-SsoI.

[0674] These results demonstrate that the recombinant vaccinia viruses are indeed carriers of the transgenes and allow the expression of the SRAS glycoprotein in its membrane form (S) or in a soluble or secreted form (SsoI). The vaccinia viruses carrying the synthetic promoter 480 allow the expression of S and the secretion of SsoI at levels much higher than the viruses carrying the promoter of the 7.5K gene.

4) Application to the Production of a Soluble Form of SARS-CoV S. Purification of this Recombinant Antigen and Diagnostic Applications

[0675] The BHK-21 line is the cell line which secretes the highest quantities of SsoI polypeptide after infection with the VV-TN-SsoI virus among the lines tested (BHK-21, CV1, 293T and FrhK-4, FIG. **36**B); it allows the quantitative production and purification of the recombinant SsoI polypeptide. In a typical experiment where the experimental conditions for infection, production and purification were optimized, the BHK-21 cells are inoculated in standard culture medium (pyruvate-free DMEM containing 4.5 g/l of glucose and supplemented with 5% TPB, 5% FCS, 100 U/ml of penicillin and 100 μg/ml of streptomycin) in the form of a subconfluent monolayer (10 million cells for each 100 cm^2 in 25 ml of medium). After 24 h of incubation at 37° C. under 5% CO_2, the cells are infected at an M.O.I. of 0.03 and the standard medium replaced with the secretion medium where the quantity of FCS is reduced to 0.5% and the TPB eliminated. The culture supernatant is removed after 2.5 days of incubation at 35° C. and under 5% CO_2 and the vaccinia virus inactivated by addition of Triton X-100 (0.1%). After filtration on 0.1 μm polyethersulfone (PES) membrane, the recombinant SsoI polypeptide is purified by

affinity chromatography on an anti-FLAG matrix with elution with a solution of FLAG peptide (DYKDDDDK) at 100 μg/ml in TBS (50 mM Tris, pH 7.4, 150 mM NaCl).

[0676] The analysis by 8% SDS acrylamide gel stained with silver nitrate identified a predominant polypeptide whose molecular mass is about 180 kD and whose degree of purity is greater than 90% (FIG. **37**). The concentration of the purified SsoI recombinant polypeptide was determined by comparison with molecular mass markers and estimated at 24 ng/μl.

[0677] This purified SsoI polypeptide preparation makes it possible to produce a calibration series in order to measure, with the aid of a capture ELISA test, the SsoI concentrations present in the culture supernatants. According to this test, the BHK-21 line secretes about 1 g/ml of SsoI polypeptide under the production conditions described above. In addition, the purification scheme presented makes it possible to purify of the order of 160 μg of SsoI polypeptide per liter of culture supernatant.

[0678] The ELISA reactivity of the recombinant SsoI polypeptide was analyzed toward sera from patients suffering from SARS.

[0679] The sera of probable cases of SARS tested were chosen on the basis of the results (positive or negative) of analysis of their specific reactivity toward the native antigens of SARS-CoV by immunofluorescence test on VeroE6 cells infected with SARS-CoV and/or by indirect ELISA test using, as antigen, a lysate of VeroE6 cells infected with SARS-CoV. The sera of these patients are identified by a serial number of the National Reference Center for Influenza Viruses and by the patient's initials and the number of days elapsed since the onset of the symptoms. All the sera of probable cases (cf. table XVI) recognize the native antigens of SARS-CoV with the exception of the serum 032552 of the patient VTT, for which infection with SARS-CoV could not be confirmed by RT-PCR performed on respiratory samples of days 3, 8 and 12. A panel of control sera was used as control (TV sera): they are sera collected in France before the SARS epidemic which occurred in 2003.

TABLE XVI

Sera of probable cases of SARS

Serum	Patient	Sample collection day
033168	JYK	38
033597	JYK	74
032632	NTM	17
032634	THA	15
032541	PHV	10
032542	NIH	17
032552	VTT	8
032633	PTU	16

[0680] Solid phases sensitized with the recombinant SsoI polypeptide were prepared by adsorption of a solution of purified SsoI polypeptide at 4 μg/ml in PBS in the wells of an ELISA plate. The plates are incubated overnight at 4° C. and then washed with PBS-Tween buffer (PBS, 0.1% Tween 20). After washing with PBS-Tween, the sera to be tested (100 μl) are diluted 1/100 and 1/400 in PBS-skimmed milk-Tween buffer (PBS, 3% skimmed milk, 0.1% Tween) and then added to the wells of the sensitized ELISA plate. The plates are then incubated for 1 h at 37° C. After 3 washings with PBS-Tween buffer, the anti-human IgG conjugate labeled with peroxidase (ref. NA933V, Amersham) diluted 1/4000 in PBS-skimmed milk-Tween buffer is added and then the plates are incubated for one hour at 37° C. After 6 washings with PBS-Tween buffer, the chromogen (TMB) and the substrate (H_2O_2) are added and the plates are incubated for 10 minutes protected from light. The reaction is stopped by adding a 1M solution of H_3PO_4 and then the absorbance is measured at 450 nm with a reference at 620 nm.

[0681] The ELISA tests (FIG. **38**) demonstrate that the recombinant SsoI polypeptide is specifically recognized by the serum antibodies of patients suffering from SARS, collected at the middle or late phase of infection (≧10 days after the onset of the symptoms), whereas it is not significantly recognized by the serum antibodies of the control sera of subjects not suffering from SARS.

[0682] In conclusion, these results demonstrate that the recombinant SsoI polypeptide can be purified from the supernatant of mammalian cells infected with the recombinant vaccinia virus W-TN-SsoI and can be used as antigen for developing an ELISA test for serological diagnosis of infection with SARS-CoV.

5. Vaccine Applications

[0683] The immunogenicity of the recombinant vaccinia viruses was studied in mice.

[0684] For that, groups of 7 BALB/c mice were immunized by the i.v. route twice at 4 weeks' interval with 10^6 p.f.u. of recombinant vaccinia viruses W-TG, VV-TG-S, W-TG-SsoI, VV-TN, VV-TN-S and W-TN-SsoI and, as a control, VV-TG-HA which directs the expression of hemagglutinin of the A/PR/8/34 strain of the influenza virus. The immune sera were collected 3 weeks after each of the immunizations (IS1, IS2).

[0685] The immune sera were analyzed per pool for each of the groups by indirect ELISA using a lysate of VeroE6 cells infected with SARS-CoV as antigen and, as control, a lysate of noninfected VeroE6 cells. The anti-SARS-CoV antibody titers (TI) are calculated as the reciprocal of the dilution producing a specific OD of 0.5 after visualization with an anti-mouse IgG(H+L) polyclonal antibody coupled with peroxidase (NA931V, Amersham) and TMB supplemented with H_2O_2 (KPL). This analysis (FIG. **39**A) shows that immunization with the virus VV-TG-S and VV-TN-S induces in mice, from the first immunization, antibodies directed against the native form of the SARS-CoV spicule protein present in the lysate of infected VeroE6 cells. The responses induced by the VV-TN-S virus are higher than those induced by the VV-TG-S virus after the first (TI=740 and TI=270 respectively) and the second (TI=3230 and TI=600 respectively) immunization. The VV-TN-SsoI virus induces high anti-SARS-CoV antibody titers after two immunizations (TI=640), whereas the virus VV-TG-SsoI induces a response at the detection limit (TI=40).

[0686] The immune sera were analyzed per pool for each of the groups for their capacity to seroneutralize the infectivity of SARS-CoV. 4 seroneutralization points on FRhK-4 cells (100 TCID50 of SARS-CoV) are produced for each of the 2-fold dilutions tested from 1/20. The seroneutralizing titer is calculated according to the Reed and Munsch method

as the reciprocal of the dilution neutralizing the infectivity of 2 wells out of 4. This analysis shows that the antibodies induced in mice by the vaccinia viruses expressing the S protein or the Ssol polypeptide are neutralizing and that the viruses with synthetic promoters are more efficient immunogens than the viruses carrying the 7.5K promoter: the highest titers (640) are observed after 2 immunizations with the virus VV-TN-S (FIG. **39**B).

[0687] The protective power of the neutralizing antibodies induced in mice after immunization with the recombinant vaccinia viruses is evaluated with the aid of a challenge infection with SARS-CoV.

6) Other Applications

[0688] Third generation recombinant vaccinia viruses are constructed by substituting the wild-type sequences of the S and Ssol genes by synthetic genes optimized for the expression in mammalian cells, described above. These recombinant vaccinia viruses are capable of expressing larger quantities of S and Ssol antigens and therefore of exhibiting increased immunogenicity.

[0689] The recombinant vaccinia virus VV-TN-Ssol can be used for the quantitative production and purification of the Ssol antigen for diagnostic (serology by ELISA) and vaccine (subunit vaccine) applications.

EXAMPLE 17

Recombinant Measles Virus Expressing the SARS-Associated Coronavirus (SARS-CoV) Spicule (S) Protein. Vaccine Applications

1) Introduction

[0690] The measles vaccine (MV) induces a lasting protective immunity in humans after a single injection (Hilleman, 2002, Vaccine, 20: 651-665). The protection conferred is very robust and is based on the induction of an antibody response and of a CD4 and CD8 cell response. The MV genome is very stable and no reversion of the vaccine strains to virulence has ever been observed. The measles virus belongs to the genus Morbillivirus of the Paramyxoviridae family; it is an enveloped virus whose genome is a 16 kb single-stranded RNA of negative polarity (FIG. **40**A) and whose exclusively cytoplasmic replication cycle excludes any possibility of integration into the genome of the host. The measles vaccine is thus one of the most effective and one of the safest live vaccines used in the human population. Frédéric Tangy's team recently developed an expression vector on the basis of the Schwarz strain of the measles virus, which is the safest attenuated strain and the most widely used in humans as vaccine against measles. This vaccine strain may be isolated from an infectious molecular clone while preserving its immuno-genicity in primates and in mice that are sensitive to the infection. It constitutes, after insertion of additional transcription units, a vector for the expression of heterologous sequences (Combredet, 2003, J. Virol. 77: 11546-11554). In addition, a recombinant MV Schwarz expressing the envelope glycoprotein of the West Nile virus (WNV) induces an effective and lasting antibody response which protects mice from a lethal challenge infection with WNV (Despres et al., 2004, J. Infect. Dis., in press). All these characteristics make the attenuated Schwarz strain of the measles virus an extremely promising candidate vector for the construction of novel recombinant live vaccines.

[0691] The aim of this example is to evaluate the capacity of recombinant measles viruses (MV) expressing various SARS-associated coronavirus (SARS-CoV) antigens to constitute novel candidate vaccines against SARS.

[0692] The inventors focused on the SARS-CoV spicule (S) protein, which makes it possible to induce, after gene immunization in animals, antibodies neutralizing the infectivity of SARS-CoV, and on a soluble and secreted form of this protein, the Ssol polypeptide, which is composed of the ectodomain (aa 1-1193) of S fused at its C-ter end with a FLAG tag (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide. This Ssol polypeptide exhibits a similar antigenicity to that of the S protein and allows, after injection into mice in the form of a purified protein adjuvanted with aluminum hydroxide, the induction of high neutralizing antibody titers against SARS-CoV.

[0693] The various forms of the S gene were introduced in the form of an additional transcription unit between the P (phosphoprotein) and M (matrix) genes into the cDNA of the Schwarz strain of Mv previously described (Combredet, 2003, J. Virol. 77: 11546-11554; EP application No. 02291551.6 of Jun. 20, 2002, and EP application No. 02291550.8 of Jun. 20, 2002). After having isolated the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and checked their capacity to express the SARS-CoV S antigen, their capacity to induce a protective immune response against SARS in mice and then in monkeys was tested.

2) Construction of the Recombinant Viruses

[0694] The plasmid pTM-MVSchw-ATU2 (FIG. **40**B) contains an infectious cDNA corresponding to the antigenome of the Schwarz vaccine strain of the measles virus (MV) into which an additional transcription unit (ATU) has been introduced between the P (phosphoprotein) and M (matrix) genes (Combredet, 2003, Journal of Virology, 77: 11546-11554). Recombinant genomes MVSchw2-SARS-S and MVSchw2-SARS-Ssol of the measles virus were constructed by inserting ORFs of the S protein and of the Ssol polypeptide into the additional transcription unit of the MVSchw-ATU2 vector.

[0695] For that, a DNA fragment containing the SARS-CoV S cDNA was amplified by PCR with the aid of the oligo-nucleotides 5'-ATACGTACGA CCATGTTTAT TTTCTTATTA TTTCTTACTC TCACT-3' and 5'-ATAGCGCGCT CATTATGTGT AATGTAATTT GACACCCTTG-3' using the plasmid pcDNA-S as template and then inserted into the plasmid pCR®2.1-TOPO (Invitrogen) in order to obtain the plasmid pTOPO-S-MV. The two oligonucleotides used contain restriction sites BsiW1 and BssHII, so as to allow subsequent insertion into the measles vector, and were designed so as to generate a sequence of 3774 nt including the codons for initiation and termination, so as to observe the rule of 6 which stipulates that the length of the genome of a measles virus must be divisible by 6 (Calain & Roux, 1993, J. Virol., 67: 4822-4830; Schneider et al., 1997, Virology, 227: 314-322). The insert was sequenced with the aid of a BigDye Terminator v1.1 kit (Applied Biosystems) and an automated sequencer ABI377.

[0696] To express a soluble and secreted form of SARS-CoV S, a plasmid containing the cDNA of the Ssol polypeptide corresponding to the ectodomain (aa 1-1193) of SARS-

CoV S fused at its C-ter end with the sequence of a FLAG tag (DYKDDDDK) via a BspE1 linker encoding the SG dipeptide was then obtained. For that, a DNA fragment was amplified with the aid of the oligonucleotides 5'-CCATTTCAAC AATTTGGCCG-3' and 5'-ATAGGATC-CGCGCGCTCATT ATTTATCGTC GTCATCTTTA TAATC-3' from the plasmid pcDNA-Ssol and then inserted into the plasmid pTOPO-S-MV between the SalI and BamH1 sites in order to obtain the plasmid pTOPO-S-MV-SF. The sequence generated is 3618 nt long between the BsiW1 and BssHII sites and observes the rule of 6. The insert was sequenced as indicated above.

[0697] The BsiW1-BssHII fragments containing the cDNAs for the S protein and the Ssol polypeptide were then excised by digestion of the plasmids pTOPO-S-MV and pTOPO-S-MV-SF and then subcloned between the corresponding sites of the plasmid pTM-MVSchw-ATU2 in order to give the plasmids pTM-MVSchw2-SARS-S and pTM-MVSchw2-SARS-Ssol (FIG. **40**B). These two plasmids were deposited at the C.N.C.M. on Dec. 1, 2004, under the numbers I-3326 and I-3327, respectively.

[0698] The recombinant measles viruses corresponding to the plasmids pTM-MVSchw2-SARS-S and pTM-MVSchw2-SARS-Ssol were obtained by reverse genetics according to the system based on the use of a helper cell line, described by Radecke et al. (1995, Embo J., 14: 5773-5784) and modified by Parks et al. (1999, J. Virol., 73: 3560-3566). Briefly, the helper cells 293-3-46 are transfected according to the calcium phosphate method with 5 μg of the plasmids pTM-MVSchw2-SARS-S or pTM-MVSchw2-SARS-Ssol and 0.02 μg of the plasmid pEMC-La directing the expression of the MV L polymerase (gift from M. A. Billeter). After incubating overnight at 37° C., a heat shock is produced for 2 hours at 43° C. and the transfected cells are transferred onto a monolayer of Vero cells. For each of the two plasmids, syncytia appeared after 2 to 3 days of coculture and were transferred successively onto monolayers of Vero cells at 70% confluence in 35 mm Petri dishes and then in 25 and 75 cm^2 flasks. When the syncytia have reached 80-90% confluence, the cells are recovered with the aid of a scraper and then frozen and thawed once. After low-speed centrifugation, the supernatant containing the virus is stored in aliquots at −80° C. The titers of the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol were determined by limiting dilution on Vero cells and the titer as dose infecting 50% of the wells ($TCID_{50}$) calculated according to the Kärber method.

3) Characterization of the Recombinant Viruses

[0699] The expression of the transgenes encoding the S protein and the Ssol polypeptide was assessed by Western blotting and immunofluorescence.

[0700] Monolayers of Vero cells in T-25 flasks were infected at a multiplicity of 0.05 by various passages of the two viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as a control. When the syncytia had reached 80 to 90% confluence, cytoplasmic extracts were prepared in an extraction buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.2, 1% Triton X-100, 0.1% SDS, 1% DOC) and then diluted in loading buffer according to Laemmli, separated on 8% SDS polyacrylamide gel and transferred onto a PVDF membrane (BioRad). The detection of this immunoblot (Western blot) was carried out with the aid of an anti-S rabbit polyclonal serum (immune serum of the rabbit P11135: cf. example 4 above) and donkey polyclonal antibodies directed against rabbit IgGs and coupled with peroxidase (NA934V, Amersham). The bound antibodies were visualized by luminescence with the aid of the ECL+ kit (Amersham) and Hyperfilm MP autoradiography films (Amersham).

[0701] Vero cells in monolayers on glass slides were infected with the two viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol and the wild-type virus MWSchw as a control at multiplicities of infection of 0.05. When the syncytia had reached 90 to 100% (MVSchw2-SARS-Ssol virus) or 30 to 40% (MVSchw2-SARS-S, MWSchw) confluence, the cells were fixed in a 4% PBS-PFA solution, permeabilized with a PBS solution containing 0.2% Triton and then labeled with rabbit polyclonal antibodies hyperimmunized with purified and inactivated SARS-CoV virions and with an anti-rabbit IgG(H+L) goat antibody conjugate coupled with FITC (Jackson).

[0702] As shown in FIGS. **41** and **42**, the recombinant viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol direct the expression of the S protein and the Ssol polypeptide respectively at levels comparable to those which can be observed 8 h after infection with SARS-CoV. The expression of these polypeptides is stable after 3 passages of the recombinant viruses in cell culture. These results demonstrate that the recombinant measles viruses are indeed carriers of the transgenes and allow the expression of the SARS glycoprotein in its membrane form (S) or in a soluble form (Ssol). The Ssol polypeptide is expected to be secreted by cells infected with the MVSchw2-SARS-Ssol virus as is the case when this same polypeptide is expressed in mammalian cells after transient transfection of the corresponding sequences (cf. example 11 above).

4) Applications

[0703] Having shown that the viruses MVSchw2-SARS-S and MVSchw2-SARS-Ssol allow the expression of the SARS-CoV S, their capacity to induce a protective immune response against SARS-CoV in $CD46^{+/-}$ IFN-$\alpha\beta R^{-/-}$ mice, which is sensitive to infection by MV, is evaluated. The antibody response of the immunized mice is evaluated by ELISA test against the native antigens of SARS-CoV and for their capacity to neutralize the infectivity of SARS-CoV in vitro, using the methodologies described above. The protective power of the response will be evaluated by measuring the reduction in the pulmonary viral load 2 days after a nonlethal challenge infection with SARS-CoV.

[0704] Second generation recombinant measles viruses are constructed by substituting the wild-type sequences of the S and Sol genes by synthetic genes optimized for expression in mammalian cells, described in example 15 above. These recombinant measles viruses are capable of expressing larger quantities of the S and Ssol antigens and therefore of exhibiting increased immunogenicity.

[0705] Alternatively, the wild-type or synthetic genes encoding the S protein or the Ssol polypeptide may be inserted into the measles vector MVSchw-ATU3 in the form of an additional transcription unit located between the H and L genes, and then the recombinant viruses produced and characterized in a similar manner. This insertion is capable of generating recombinant viruses possessing different char-

acteristics (multiplication of the virus, level of expression of the transgene) and possibly an improved immunogenicity compared with those obtained after insertion of the transgenes between the P and N genes.

[0706] The recombinant measles virus MVSchw2-SARS-Ssol may be used for the quantitative production and the purification of the Ssol antigen for diagnostic and vaccine applications.

EXAMPLE 18

Other Applications Linked to the S Protein

[0707] a) The lentiviral vectors allowing the expression of S or Ssol (or even of fragments of S) can constitute a recombinant vaccine against SARS-CoV, to be used in human or veterinary prophylaxis. In order to demonstrate the feasibility of such a vaccine, the immunogenicity of the recombinant lentiviral vectors TRIP-SD/SA-S-WPRE and TRIP-SD/SA-Ssol-WPRE is studied in mice.

[0708] b) Monoclonal antibodies are produced with the aid of the recombinant Ssol polypeptide. According to the results presented in example 14 above, these antibodies or at least the majority of them will recognize the native form of the SARS-CoV S and will be capable of diagnostic and/or prophylactic applications.

[0709] c) A serological test for SARS is developed with the Ssol polypeptide used as antigen and the double epitope methodology.

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Tyr Tyr Pro Asp Glu Ile Phe Arg Ser Asp Thr Leu Tyr Leu Thr Gln
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Thr Asn Val Val Ile Arg Ala Cys Asn Phe Glu Leu Cys Asp Asn Pro
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Ser Leu Asp Val Ser Glu Lys Ser Gly Asn Phe Lys His Leu Arg Glu
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Gln Pro Ile Asp Val Val Arg Asp Leu Pro Ser Gly Phe Asn Thr Leu
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Lys Pro Ile Phe Lys Leu Pro Leu Gly Ile Asn Ile Thr Asn Phe Arg
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Ala Ile Leu Thr Ala Phe Ser Pro Ala Gln Asp Ile Trp Gly Thr Ser
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Ala Ala Ala Tyr Phe Val Gly Tyr Leu Lys Pro Thr Thr Phe Met Leu
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Lys Tyr Asp Glu Asn Gly Thr Ile Thr Asp Ala Val Asp Cys Ser Gln
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Asn Pro Leu Ala Glu Leu Lys Cys Ser Val Lys Ser Phe Glu Ile Asp
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Val Val Arg Phe Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val
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Leu Cys Phe Ser Asn Val Tyr Ala Asp Ser Phe Val Val Lys Gly Asp
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Arg Tyr Leu Arg His Gly Lys Leu Arg Pro Phe Glu Arg Asp Ile Ser
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Asn Val Pro Phe Ser Pro Asp Gly Lys Pro Cys Thr Pro Pro Ala Leu
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Asn Ala Pro Ala Thr Val Cys Gly Pro Lys Leu Ser Thr Asp Leu Ile
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Val Leu Thr Pro Ser Ser Lys Arg Phe Gln Pro Phe Gln Gln Phe Gly
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cgt gat gtt tct gat ttc act gat tcc gtt cga gat cct aaa aca tct     1792
Arg Asp Val Ser Asp Phe Thr Asp Ser Val Arg Asp Pro Lys Thr Ser
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Glu Ile Leu Asp Ile Ser Pro Cys Ser Phe Gly Gly Val Ser Val Ile
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Thr Pro Gly Thr Asn Ala Ser Ser Glu Val Ala Val Leu Tyr Gln Asp
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Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala Ser Tyr His Thr Val Ser
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Arg Glu Val Phe Ala Gln Val Lys Gln Met Tyr Lys Thr Pro Thr Leu
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agc ctt caa acc tat gta aca caa caa cta atc agg gct gct gaa atc     3088
Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile
985                 990                 995                 1000

agg gct tct gct aat  ctt gct gct act aaa  atg tct gag tgt gtt       3133
Arg Ala Ser Ala Asn  Leu Ala Ala Thr Lys  Met Ser Glu Cys Val
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                1005                1010                1015

ctt gga caa tca aaa  aga gtt gac ttt tgt  gga aag ggc tac cac       3178
Leu Gly Gln Ser Lys  Arg Val Asp Phe Cys  Gly Lys Gly Tyr His
                1020                1025                1030

ctt atg tcc ttc cca  caa gca gcc ccg cat  ggt gtt gtc ttc cta       3223
Leu Met Ser Phe Pro  Gln Ala Ala Pro His  Gly Val Val Phe Leu
                1035                1040                1045

cat gtc acg tat gtg  cca tcc cag gag agg  aac ttc acc aca gcg       3268
His Val Thr Tyr Val  Pro Ser Gln Glu Arg  Asn Phe Thr Thr Ala
                1050                1055                1060

cca gca att tgt cat  gaa ggc aaa gca tac  ttc cct cgt gaa ggt       3313
Pro Ala Ile Cys His  Glu Gly Lys Ala Tyr  Phe Pro Arg Glu Gly
                1065                1070                1075

gtt ttt gtg ttt aat  ggc act tct tgg ttt  att aca cag agg aac       3358
Val Phe Val Phe Asn  Gly Thr Ser Trp Phe  Ile Thr Gln Arg Asn
                1080                1085                1090

ttc ttt tct cca caa  ata att act aca gac  aat aca ttt gtc tca       3403
Phe Phe Ser Pro Gln  Ile Ile Thr Thr Asp  Asn Thr Phe Val Ser
                1095                1100                1105

gga aat tgt gat gtc  gtt att ggc atc att  aac aac aca gtt tat       3448
Gly Asn Cys Asp Val  Val Ile Gly Ile Ile  Asn Asn Thr Val Tyr
                1110                1115                1120

gat cct ctg caa cct  gag ctt gac tca ttc  aaa gaa gag ctg gac       3493
Asp Pro Leu Gln Pro  Glu Leu Asp Ser Phe  Lys Glu Glu Leu Asp
                1125                1130                1135

aag tac ttc aaa aat  cat aca tca cca gat  gtt gat ctt ggc gac       3538
Lys Tyr Phe Lys Asn  His Thr Ser Pro Asp  Val Asp Leu Gly Asp
                1140                1145                1150

att tca ggc att aac  gct tct gtc gtc aac  att caa aaa gaa att       3583
Ile Ser Gly Ile Asn  Ala Ser Val Val Asn  Ile Gln Lys Glu Ile
                1155                1160                1165

gac cgc ctc aat gag  gtc gct aaa aat tta  aat gaa tca ctc att       3628
Asp Arg Leu Asn Glu  Val Ala Lys Asn Leu  Asn Glu Ser Leu Ile
                1170                1175                1180

gac ctt caa gaa ttg  gga aaa tat gag caa  tat att aaa tgg cct       3673
Asp Leu Gln Glu Leu  Gly Lys Tyr Glu Gln  Tyr Ile Lys Trp Pro
                1185                1190                1195

tgg tat gtt tgg ctc  ggc ttc att gct gga  cta att gcc atc gtc       3718
Trp Tyr Val Trp Leu  Gly Phe Ile Ala Gly  Leu Ile Ala Ile Val
                1200                1205                1210

atg gtt aca atc ttg  ctt tgt tgc atg act  agt tgt tgc agt tgc       3763
Met Val Thr Ile Leu  Leu Cys Cys Met Thr  Ser Cys Cys Ser Cys
                1215                1220                1225

ctc aag ggt gca tgc  tct tgt ggt tct tgc  tgc aag ttt gat gag       3808
Leu Lys Gly Ala Cys  Ser Cys Gly Ser Cys  Cys Lys Phe Asp Glu
                1230                1235                1240

gat gac tct gag cca  gtt ctc aag ggt gtc  aaa tta cat tac aca       3853
Asp Asp Ser Glu Pro  Val Leu Lys Gly Val  Lys Leu His Tyr Thr
                1245                1250                1255

taaacgaact tatggatttg tttatgagat tttttactct tggatcaatt actgcacagc   3913

cagtaaaaat tgacaatgct tctcctgcaa gt                                 3945
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<210> SEQ ID NO 3
<211> LENGTH: 1255
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

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<400> SEQUENCE: 3

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Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1               5                  10                  15

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
            20                  25                  30

His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
        35                  40                  45

Ser Asp Thr Leu Tyr Leu Thr Gln Asp Leu Phe Leu Pro Phe Tyr Ser
    50                  55                  60

Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65                  70                  75                  80

Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
                85                  90                  95

Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
            100                 105                 110

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
        115                 120                 125

Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
    130                 135                 140

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145                 150                 155                 160

Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
                165                 170                 175

Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
            180                 185                 190

Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
        195                 200                 205

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
    210                 215                 220

Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225                 230                 235                 240

Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
                245                 250                 255

Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
            260                 265                 270

Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
        275                 280                 285

Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
    290                 295                 300

Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
305                 310                 315                 320

Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser
                325                 330                 335

Val Tyr Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr
            340                 345                 350

Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
        355                 360                 365

Val Ser Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala
    370                 375                 380

Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
385                 390                 395                 400
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Gln Thr Gly Val Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe
                405                 410                 415

Met Gly Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser
            420                 425                 430

Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
        435                 440                 445

Arg Pro Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly
    450                 455                 460

Lys Pro Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp
465                 470                 475                 480

Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
                485                 490                 495

Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
            500                 505                 510

Pro Lys Leu Ser Thr Asp Leu Ile Lys Asn Gln Cys Val Asn Phe Asn
        515                 520                 525

Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Pro Ser Ser Lys Arg
    530                 535                 540

Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
545                 550                 555                 560

Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
                565                 570                 575

Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
            580                 585                 590

Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
        595                 600                 605

Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
    610                 615                 620

Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625                 630                 635                 640

His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
                645                 650                 655

Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
            660                 665                 670

Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
        675                 680                 685

Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
    690                 695                 700

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705                 710                 715                 720

Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
                725                 730                 735

Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
            740                 745                 750

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
        755                 760                 765

Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
    770                 775                 780

Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785                 790                 795                 800
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Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
                805                 810                 815

Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
            820                 825                 830

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
        835                 840                 845

Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
    850                 855                 860

Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
865                 870                 875                 880

Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr Gln Asn
                885                 890                 895

Val Leu Tyr Glu Asn Gln Lys Gln Ile Ala Asn Gln Phe Asn Lys Ala
            900                 905                 910

Ile Ser Gln Ile Gln Glu Ser Leu Thr Thr Thr Ser Thr Ala Leu Gly
        915                 920                 925

Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn Thr Leu
    930                 935                 940

Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val Leu Asn
945                 950                 955                 960

Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln Ile Asp
                965                 970                 975

Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val Thr Gln
            980                 985                 990

Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn Leu Ala Ala
        995                 1000                1005

Thr Lys  Met Ser Glu Cys Val  Leu Gly Gln Ser Lys  Arg Val Asp
    1010                1015                1020

Phe Cys  Gly Lys Gly Tyr His  Leu Met Ser Phe Pro  Gln Ala Ala
    1025                1030                1035

Pro His  Gly Val Val Phe Leu  His Val Thr Tyr Val  Pro Ser Gln
    1040                1045                1050

Glu Arg  Asn Phe Thr Thr Ala  Pro Ala Ile Cys His  Glu Gly Lys
    1055                1060                1065

Ala Tyr  Phe Pro Arg Glu Gly  Val Phe Val Phe Asn  Gly Thr Ser
    1070                1075                1080

Trp Phe  Ile Thr Gln Arg Asn  Phe Phe Ser Pro Gln  Ile Ile Thr
    1085                1090                1095

Thr Asp  Asn Thr Phe Val Ser  Gly Asn Cys Asp Val  Val Ile Gly
    1100                1105                1110

Ile Ile  Asn Asn Thr Val Tyr  Asp Pro Leu Gln Pro  Glu Leu Asp
    1115                1120                1125

Ser Phe  Lys Glu Glu Leu Asp  Lys Tyr Phe Lys Asn  His Thr Ser
    1130                1135                1140

Pro Asp  Val Asp Leu Gly Asp  Ile Ser Gly Ile Asn  Ala Ser Val
    1145                1150                1155

Val Asn  Ile Gln Lys Glu Ile  Asp Arg Leu Asn Glu  Val Ala Lys
    1160                1165                1170

Asn Leu  Asn Glu Ser Leu Ile  Asp Leu Gln Glu Leu  Gly Lys Tyr
    1175                1180                1185

Glu Gln  Tyr Ile Lys Trp Pro  Trp Tyr Val Trp Leu  Gly Phe Ile
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    1190                1195                1200

Ala Gly  Leu Ile Ala Ile Val  Met Val Thr Ile Leu  Leu Cys Cys
    1205                1210                1215

Met Thr  Ser Cys Cys Ser Cys  Leu Lys Gly Ala Cys  Ser Cys Gly
    1220                1225                1230

Ser Cys  Cys Lys Phe Asp Glu  Asp Asp Ser Glu Pro  Val Leu Lys
    1235                1240                1245

Gly Val  Lys Leu His Tyr Thr
    1250                1255
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<210> SEQ ID NO 4
<211> LENGTH: 3943
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 4

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ctcttctgga aaaaggtagg cttatcatta gagaaaacaa cagagttgtg gtttcaagtg     60

atattcttgt taacaactaa acgaacatgt ttattttctt attatttctt actctcacta    120

gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc    180

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc    240

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta    300

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca    360

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac    420

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat    480

tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga    540

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg    600

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg    660

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg    720

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta    780

gagccattct tacagccttt tcacctgctc aagacatttg gggcacgtca gctgcagcct    840

attttgttgg ctatttaaag ccaactacat ttatgctcaa gtatgatgaa aatggtacaa    900

tcacagatgc tgttgattgt tctcaaaatc cacttgctga actcaaatgc tctgttaaga    960

gctttgagat tgacaaagga atttaccaga cctctaattt cagggttgtt ccctcaggag   1020

atgttgtgag attccctaat attacaaact tgtgtccttt tggagaggtt tttaatgcta   1080

ctaaattccc ttctgtctat gcatgggaga gaaaaaaaat ttctaattgt gttgctgatt   1140

actctgtgct ctacaactca acatttttt caacctttaa gtgctatggc gtttctgcca   1200

ctaagttgaa tgatctttgc ttctccaatg tctatgcaga ttcttttgta gtcaagggag   1260

atgatgtaag acaaatagcg ccaggacaaa ctggtgttat tgctgattat aattataaat   1320

tgccagatga tttcatgggt tgtgtccttg cttggaatac taggaacatt gatgctactt   1380

caactggtaa ttataattat aaatataggt atcttagaca tggcaagctt aggccctttg   1440

agagagacat atctaatgtg cctttctccc ctgatggcaa accttgcacc ccacctgctc   1500

ttaattgtta ttggccatta aatgattatg gtttttacac cactactggc attggctacc   1560

aaccttacag agttgtagta ctttcttttg aacttttaaa tgcaccggcc acggtttgtg   1620
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gaccaaaatt atccactgac cttattaaga accagtgtgt caattttaat tttaatggac 1680

tcactggtac tggtgtgtta actccttctt caaagagatt tcaaccattt caacaatttg 1740

gccgtgatgt ctctgatttc actgattccg ttcgagatcc taaaacatct gaaatattag 1800

acatttcacc ttgctctttt gggggtgtaa gtgtaattac acctggaaca aatgcttcat 1860

ctgaagttgc tgttctatat caagatgtta actgcactga tgtttctaca gcaatccatg 1920

cagatcaact cacaccagct tggcgcatat attctactgg aaacaatgta ttccagactc 1980

aagcaggctg tcttatagga gctgagcatg tcgacacttc ttatgagtgc gacattccta 2040

ttggagctgg catttgtgct agttaccata cagtttcttt attacgtagt actagccaaa 2100

aatctattgt ggcttatact atgtctttag gtgctgatag ttcaattgct tactctaata 2160

acaccattgc tatacctact aacttttcaa ttagcattac tacagaagta atgcctgttt 2220

ctatggctaa aacctccgta gattgtaata tgtacatctg cggagattct actgaatgtg 2280

ctaatttgct tctccaatat ggtagctttt gcacacaact aaatcgtgca ctctcaggta 2340

ttgctgctga acaggatcgc aacacacgtg aagtgttcgc tcaagtcaaa caaatgtaca 2400

aaaccccaac tttgaaatat tttggtggtt ttaatttttc acaaatatta cctgaccctc 2460

taaagccaac taagaggtct tttattgagg acttgctctt taataaggtg acactcgctg 2520

atgctggctt catgaagcaa tatggcgaat gcctaggtga tattaatgct agagatctca 2580

tttgtgcgca gaagttcaat gggcttacag tgttgccacc tctgctcact gatgatatga 2640

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ctggcgctgc tcttcaaata ccttttgcta tgcaaatggc atataggttc aatggcattg 2760

gagttaccca aaatgttctc tatgagaacc aaaaacaaat cgccaaccaa tttaacaagg 2820

cgattagtca aattcaagaa tcacttacaa caacatcaac tgcattgggc aagctgcaag 2880

acgttgttaa ccagaatgct caagcattaa acacacttgt taaacaactt agctctaatt 2940

ttggtgcaat ttcaagtgtg ctaaatgata tcctttcgcg acttgataaa gtcgaggcgg 3000

aggtacaaat tgacaggcta attacaggca gacttcaaag ccttcaaacc tatgtaacac 3060

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cccaggagag gaacttcacc acagcgccag caatttgtca tgaaggcaaa gcatacttcc 3300

ctcgtgaagg tgtttttgtg tttaatggca cttcttggtt tattacacag aggaacttct 3360

tttctccaca aataattact acagacaata catttgtctc aggaaattgt gatgtcgtta 3420

ttggcatcat taacaacaca gtttatgatc ctctgcaacc tgagcttgac tcattcaaag 3480

aagagctgga caagtacttc aaaaatcata catcaccaga tgttgatctt ggcgacattt 3540

caggcattaa cgcttctgtc gtcaacattc aaaaagaaat tgaccgcctc aatgaggtcg 3600

ctaaaaattt aaatgaatca ctcattgacc ttcaagaatt gggaaaatat gagcaatata 3660

ttaaatggcc ttggtatgtt tggctcggct tcattgctgg actaattgcc atcgtcatgg 3720

ttacaatctt gctttgttgc atgactagtt gttgcagttg cctcaagggt gcatgctctt 3780

gtggttcttg ctgcaagttt gatgaggatg actctgagcc agttctcaag ggtgtcaaat 3840

tacattacac ataaacgaac ttatggattt gtttatgaga ttttttactc ttggatcaat 3900

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tactgcacag ccagtaaaaa ttgacaatgc ttctcctgca agt 3943

<210> SEQ ID NO 5
<211> LENGTH: 2049
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 5

ctcttctgga aaaaggtagg cttatcatta gagaaaacaa cagagttgtg gtttcaagtg 60

atattcttgt taacaactaa acgaacatgt ttattttctt attatttctt actctcacta 120

gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc 180

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc 240

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta 300

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca 360

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac 420

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat 480

tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga 540

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg 600

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg 660

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg 720

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta 780

gagccattct tacagccttt tcacctgctc aagacatttg gggcacgtca gctgcagcct 840

attttgttgg ctatttaaag ccaactacat ttatgctcaa gtatgatgaa aatggtacaa 900

tcacagatgc tgttgattgt tctcaaaatc cacttgctga actcaaatgc tctgttaaga 960

gctttgagat tgacaaagga atttaccaga cctctaattt cagggttgtt ccctcaggag 1020

atgttgtgag attccctaat attacaaact tgtgtccttt tggagaggtt tttaatgcta 1080

ctaaattccc ttctgtctat gcatgggaga gaaaaaaaat ttctaattgt gttgctgatt 1140

actctgtgct ctacaactca acatttttt caacctttaa gtgctatggc gtttctgcca 1200

ctaagttgaa tgatctttgc ttctccaatg tctatgcaga ttcttttgta gtcaagggag 1260

atgatgtaag acaaatagcg ccaggacaaa ctggtgttat tgctgattat aattataaat 1320

tgccagatga tttcatgggt tgtgtccttg cttggaatac taggaacatt gatgctactt 1380

caactggtaa ttataattat aaatataggt atcttagaca tggcaagctt aggccctttg 1440

agagagacat atctaatgtg cctttctccc ctgatggcaa accttgcacc ccacctgctc 1500

ttaattgtta ttggccatta aatgattatg gtttttacac cactactggc attggctacc 1560

aaccttacag agttgtagta ctttcttttg aacttttaaa tgcaccggcc acggtttgtg 1620

gaccaaaatt atccactgac cttattaaga accagtgtgt caattttaat tttaatggac 1680

tcactggtac tggtgtgtta actccttctt caaagagatt tcaaccattt caacaatttg 1740

gccgtgatgt ctctgatttc actgattccg ttcgagatcc taaaacatct gaaatattag 1800

acatttcacc ttgctctttt gggggtgtaa gtgtaattac acctggaaca aatgcttcat 1860

ctgaagttgc tgttctatat caagatgtta actgcactga tgtttctaca gcaatccatg 1920

cagatcaact cacaccagct tggcgcatat attctactgg aaacaatgta ttccagactc 1980

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ttggagctg 2049

<210> SEQ ID NO 6
<211> LENGTH: 2027
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 6

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actcaagcag gctgtcttat aggagctgag catgtcgaca cttcttatga gtgcgacatt 120

cctattggag ctggcatttg tgctagttac catacagttt ctttattacg tagtactagc 180

caaaaatcta ttgtggctta tactatgtct ttaggtgctg atagttcaat tgcttactct 240

aataacacca ttgctatacc tactaacttt tcaattagca ttactacaga agtaatgcct 300

gtttctatgg ctaaaacctc cgtagattgt aatatgtaca tctgcggaga ttctactgaa 360

tgtgctaatt tgcttctcca atatggtagc ttttgcacac aactaaatcg tgcactctca 420

ggtattgctg ctgaacagga tcgcaacaca cgtgaagtgt tcgctcaagt caaacaaatg 480

tacaaaaccc caactttgaa atattttggt ggttttaatt tttcacaaat attacctgac 540

cctctaaagc caactaagag gtcttttatt gaggacttgc tctttaataa ggtgacactc 600

gctgatgctg gcttcatgaa gcaatatggc gaatgcctag gtgatattaa tgctagagat 660

ctcatttgtg cgcagaagtt caatgggctt acagtgttgc cacctctgct cactgatgat 720

atgattgctg cctacactgc tgctctagtt agtggtactg ccactgctgg atggacattt 780

ggtgctggcg ctgctcttca aataccttttt gctatgcaaa tggcatatag gttcaatggc 840

attggagtta cccaaaatgt tctctatgag aaccaaaaac aaatcgccaa ccaatttaac 900

aaggcgatta gtcaaattca agaatcactt acaacaacat caactgcatt gggcaagctg 960

caagacgttg ttaaccagaa tgctcaagca ttaaacacac ttgttaaaca acttagctct 1020

aattttggtg caatttcaag tgtgctaaat gatatccttt cgcgacttga taaagtcgag 1080

gcggaggtac aaattgacag gttaattaca ggcagacttc aaagccttca aacctatgta 1140

acacaacaac taatcagggc tgctgaaatc agggcttctg ctaatcttgc tgctactaaa 1200

atgtctgagt gtgttcttgg acaatcaaaa agagttgact tttgtggaaa gggctaccac 1260

cttatgtcct tcccacaagc agccccgcat ggtgttgtct tcctacatgt cacgtatgtg 1320

ccatcccagg agaggaactt caccacagcg ccagcaattt gtcatgaagg caaagcatac 1380

ttccctcgtg aaggtgtttt tgtgtttaat ggcacttctt ggtttattac acagaggaac 1440

ttcttttctc cacaaataat tactacagac aatacatttg tctcaggaaa ttgtgatgtc 1500

gttattggcg tcattaacaa cacagtttat gatcctctgc aacctgagct tgactcattc 1560

aaagaagagc tggacaagta cttcaaaaat catacatcac cagatgttga tcttggcgac 1620

atttcaggca ttaacgcttc tgtcgtcaac attcaaaaag aaattgaccg cctcaatgag 1680

gtcgctaaaa atttaaatga atcactcatt gaccttcaag aattgggaaa atatgagcaa 1740

tatattaaat ggccttggta tgtttggctc ggcttcattg ctggactaat tgccatcgtc 1800

atggttacaa tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc 1860

tcttgtggtt cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc 1920

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aaattacatt acacataaac gaacttatgg atttgtttat gagatttttt actcttggat 1980

caattactgc acagccagta aaaattgaca atgcttctcc tgcaagt 2027

<210> SEQ ID NO 7
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 7

tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc tcttgtggtt 60

cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc aaattacatt 120

acacataaac gaacttatgg atttgtttat gagatttttt actcttggat caattactgc 180

acagccagta aaaattgaca atgcttctcc tgcaagtact gttcatgcta cagcaacgat 240

accgctacaa gcctcactcc ctttcggatg gcttgttatt ggcgttgcat ttcttgctgt 300

ttttcagagc gctaccaaaa taattgcgct caataaaaga tggcagctag ccctttataa 360

gggcttccag ttcatttgca atttactgct gctatttgtt accatctatt cacatctttt 420

gcttgtcgct gcaggtatgg aggcgcaatt tttgtacctc tatgccttga tatattttct 480

acaatgcatc aacgcatgta gaattattat gagatgttgg ctttgttgga agtgcaaatc 540

caagaaccca ttactttatg atgccaacta ctttgtttgc tggcacacac ataactatga 600

ctactgtata ccatataaca gtgtcacaga tacaattgtc gttactgaag gtgacggcat 660

ttcaacacca aaactcaaag aagactacca aattggtggt tattctgagg ataggcactc 720

aggtgttaaa gactatgtcg ttgtacatgg ctatttcacc gaagtttact accagcttga 780

gtctacacaa attactacag acactggtat tgaaaatgct acattcttca tctttaacaa 840

gcttgttaaa gacccaccga atgtgcaaat acacacaatc gacggctctt caggagttgc 900

taatccagca atggatccaa tttatgatga gccgacgacg actactagcg tgcctttgta 960

agcacaagaa agtgagtacg aacttatgta ctcattcgtt tcggaagaaa caggtacgtt 1020

aatagttaat agcgtacttc tttttcttgc tttcgtggta ttcttgctag tcacactagc 1080

catccttact gcgctt 1096

<210> SEQ ID NO 8
<211> LENGTH: 1135
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 8

attgccatcg tcatggttac aatcttgctt tgttgcatga ctagttgttg cagttgcctc 60

aagggtgcat gctcttgtgg ttcttgctgc aagtttgatg aggatgactc tgagccagtt 120

ctcaagggtg tcaaattaca ttacacataa acgaacttat ggatttgttt atgagatttt 180

ttactcttgg atcaattact gcacagccag taaaaattga caatgcttct cctgcaagta 240

ctgttcatgc tacagcaacg ataccgctac aagcctcact ccctttcgga tggcttgtta 300

ttggcgttgc atttcttgct gtttttcaga gcgctaccaa aataattgcg ctcaataaaa 360

gatggcagct agcccttat aagggcttcc agttcatttg caatttactg ctgctatttg 420

ttaccatcta ttcacatctt ttgcttgtcg ctgcaggtat ggaggcgcaa tttttgtacc 480

tctatgcctt gatatatttt ctacaatgca tcaacgcatg tagaattatt atgagatgtt 540

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ggctttgttg gaagtgcaaa tccaagaacc cattacttta tgatgccaac tactttgttt    600

gctggcacac acataactat gactactgta taccatataa cagtgtcaca gatacaattg    660

tcgttactga aggtgacggc atttcaacac caaaactcaa agaagactac caaattggtg    720

gttattctga ggataggcac tcaggtgtta aagactatgt cgttgtacat ggctatttca    780

ccgaagttta ctaccagctt gagtctacac aaattactac agacactggt attgaaaatg    840

ctacattctt catctttaac aagcttgtta aagacccacc gaatgtgcaa atacacacaa    900

tcgacggctc ttcaggagtt gctaatccag caatggatcc aatttatgat gagccgacga    960

cgactactag cgtgcctttg taagcacaag aaagtgagta cgaacttatg tactcattcg   1020

tttcggaaga aacaggtacg ttaatagtta atagcgtact tctttttctt gctttcgtgg   1080

tattcttgct agtcacacta gccatcctta ctgcgcttcg attgtgtgcg tactg        1135


<210> SEQ ID NO 9
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (137)..(958)
<223> OTHER INFORMATION:

<400> SEQUENCE: 9

tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc tcttgtggtt     60

cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc aaattacatt    120

acacataaac gaactt atg gat ttg ttt atg aga ttt ttt act ctt gga tca    172
                 Met Asp Leu Phe Met Arg Phe Phe Thr Leu Gly Ser
                 1               5                  10

att act gca cag cca gta aaa att gac aat gct tct cct gca agt act      220
Ile Thr Ala Gln Pro Val Lys Ile Asp Asn Ala Ser Pro Ala Ser Thr
        15                  20                  25

gtt cat gct aca gca acg ata ccg cta caa gcc tca ctc cct ttc gga      268
Val His Ala Thr Ala Thr Ile Pro Leu Gln Ala Ser Leu Pro Phe Gly
    30                  35                  40

tgg ctt gtt att ggc gtt gca ttt ctt gct gtt ttt cag agc gct acc      316
Trp Leu Val Ile Gly Val Ala Phe Leu Ala Val Phe Gln Ser Ala Thr
45                  50                  55                  60

aaa ata att gcg ctc aat aaa aga tgg cag cta gcc ctt tat aag ggc      364
Lys Ile Ile Ala Leu Asn Lys Arg Trp Gln Leu Ala Leu Tyr Lys Gly
                65                  70                  75

ttc cag ttc att tgc aat tta ctg ctg cta ttt gtt acc atc tat tca      412
Phe Gln Phe Ile Cys Asn Leu Leu Leu Leu Phe Val Thr Ile Tyr Ser
            80                  85                  90

cat ctt ttg ctt gtc gct gca ggt atg gag gcg caa ttt ttg tac ctc      460
His Leu Leu Leu Val Ala Ala Gly Met Glu Ala Gln Phe Leu Tyr Leu
        95                  100                 105

tat gcc ttg ata tat ttt cta caa tgc atc aac gca tgt aga att att      508
Tyr Ala Leu Ile Tyr Phe Leu Gln Cys Ile Asn Ala Cys Arg Ile Ile
    110                 115                 120

atg aga tgt tgg ctt tgt tgg aag tgc aaa tcc aag aac cca tta ctt      556
Met Arg Cys Trp Leu Cys Trp Lys Cys Lys Ser Lys Asn Pro Leu Leu
125                 130                 135                 140

tat gat gcc aac tac ttt gtt tgc tgg cac aca cat aac tat gac tac      604
Tyr Asp Ala Asn Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr
                145                 150                 155
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tgt ata cca tat aac agt gtc aca gat aca att gtc gtt act gaa ggt      652
Cys Ile Pro Tyr Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly
            160                 165                 170

gac ggc att tca aca cca aaa ctc aaa gaa gac tac caa att ggt ggt      700
Asp Gly Ile Ser Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly
        175                 180                 185

tat tct gag gat agg cac tca ggt gtt aaa gac tat gtc gtt gta cat      748
Tyr Ser Glu Asp Arg His Ser Gly Val Lys Asp Tyr Val Val Val His
    190                 195                 200

ggc tat ttc acc gaa gtt tac tac cag ctt gag tct aca caa att act      796
Gly Tyr Phe Thr Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr
205                 210                 215                 220

aca gac act ggt att gaa aat gct aca ttc ttc atc ttt aac aag ctt      844
Thr Asp Thr Gly Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu
                225                 230                 235

gtt aaa gac cca ccg aat gtg caa ata cac aca atc gac ggc tct tca      892
Val Lys Asp Pro Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser
            240                 245                 250

gga gtt gct aat cca gca atg gat cca att tat gat gag ccg acg acg      940
Gly Val Ala Asn Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr
        255                 260                 265

act act agc gtg cct ttg taagcacaag aaagtgagta cgaacttatg             988
Thr Thr Ser Val Pro Leu
    270

tactcattcg tttcggaaga aacaggtacg ttaatagtta atagcgtact tctttttctt   1048

gctttcgtgg tattcttgct agtcacacta gccatcctta ctgcgctt               1096


<210> SEQ ID NO 10
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 10

Met Asp Leu Phe Met Arg Phe Phe Thr Leu Gly Ser Ile Thr Ala Gln
1               5                   10                  15

Pro Val Lys Ile Asp Asn Ala Ser Pro Ala Ser Thr Val His Ala Thr
            20                  25                  30

Ala Thr Ile Pro Leu Gln Ala Ser Leu Pro Phe Gly Trp Leu Val Ile
        35                  40                  45

Gly Val Ala Phe Leu Ala Val Phe Gln Ser Ala Thr Lys Ile Ile Ala
    50                  55                  60

Leu Asn Lys Arg Trp Gln Leu Ala Leu Tyr Lys Gly Phe Gln Phe Ile
65                  70                  75                  80

Cys Asn Leu Leu Leu Leu Phe Val Thr Ile Tyr Ser His Leu Leu Leu
                85                  90                  95

Val Ala Ala Gly Met Glu Ala Gln Phe Leu Tyr Leu Tyr Ala Leu Ile
            100                 105                 110

Tyr Phe Leu Gln Cys Ile Asn Ala Cys Arg Ile Ile Met Arg Cys Trp
        115                 120                 125

Leu Cys Trp Lys Cys Lys Ser Lys Asn Pro Leu Leu Tyr Asp Ala Asn
    130                 135                 140

Tyr Phe Val Cys Trp His Thr His Asn Tyr Asp Tyr Cys Ile Pro Tyr
145                 150                 155                 160

Asn Ser Val Thr Asp Thr Ile Val Val Thr Glu Gly Asp Gly Ile Ser
                165                 170                 175
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Thr Pro Lys Leu Lys Glu Asp Tyr Gln Ile Gly Gly Tyr Ser Glu Asp
            180                 185                 190

Arg His Ser Gly Val Lys Asp Tyr Val Val Val His Gly Tyr Phe Thr
        195                 200                 205

Glu Val Tyr Tyr Gln Leu Glu Ser Thr Gln Ile Thr Thr Asp Thr Gly
    210                 215                 220

Ile Glu Asn Ala Thr Phe Phe Ile Phe Asn Lys Leu Val Lys Asp Pro
225                 230                 235                 240

Pro Asn Val Gln Ile His Thr Ile Asp Gly Ser Ser Gly Val Ala Asn
                245                 250                 255

Pro Ala Met Asp Pro Ile Tyr Asp Glu Pro Thr Thr Thr Thr Ser Val
            260                 265                 270

Pro Leu
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<210> SEQ ID NO 11
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (558)..(1019)
<223> OTHER INFORMATION:

<400> SEQUENCE: 11

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tcttgctttg ttgcatgact agttgttgca gttgcctcaa gggtgcatgc tcttgtggtt     60

cttgctgcaa gtttgatgag gatgactctg agccagttct caagggtgtc aaattacatt    120

acacataaac gaacttatgg atttgtttat gagatttttt actcttggat caattactgc    180

acagccagta aaaattgaca atgcttctcc tgcaagtact gttcatgcta cagcaacgat    240

accgctacaa gcctcactcc ctttcggatg gcttgttatt ggcgttgcat ttcttgctgt    300

ttttcagagc gctaccaaaa taattgcgct caataaaaga tggcagctag ccctttataa    360

gggcttccag ttcatttgca atttactgct gctatttgtt accatctatt cacatctttt    420

gcttgtcgct gcaggtatgg aggcgcaatt tttgtacctc tatgccttga tatattttct    480

acaatgcatc aacgcatgta gaattattat gagatgttgg ctttgttgga agtgcaaatc    540

caagaaccca ttacttt atg atg cca act act ttg ttt gct ggc aca cac        590
                  Met Met Pro Thr Thr Leu Phe Ala Gly Thr His
                  1               5                   10

ata act atg act act gta tac cat ata aca gtg tca cag ata caa ttg      638
Ile Thr Met Thr Thr Val Tyr His Ile Thr Val Ser Gln Ile Gln Leu
            15                  20                  25

tcg tta ctg aag gtg acg gca ttt caa cac caa aac tca aag aag act      686
Ser Leu Leu Lys Val Thr Ala Phe Gln His Gln Asn Ser Lys Lys Thr
        30                  35                  40

acc aaa ttg gtg gtt att ctg agg ata ggc act cag gtg tta aag act      734
Thr Lys Leu Val Val Ile Leu Arg Ile Gly Thr Gln Val Leu Lys Thr
    45                  50                  55

atg tcg ttg tac atg gct att tca ccg aag ttt act acc agc ttg agt      782
Met Ser Leu Tyr Met Ala Ile Ser Pro Lys Phe Thr Thr Ser Leu Ser
60                  65                  70                  75

cta cac aaa tta cta cag aca ctg gta ttg aaa atg cta cat tct tca      830
Leu His Lys Leu Leu Gln Thr Leu Val Leu Lys Met Leu His Ser Ser
                80                  85                  90

tct tta aca agc ttg tta aag acc cac cga atg tgc aaa tac aca caa      878
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Ser Leu Thr Ser Leu Leu Lys Thr His Arg Met Cys Lys Tyr Thr Gln
            95                  100                 105

tcg acg gct ctt cag gag ttg cta atc cag caa tgg atc caa ttt atg      926
Ser Thr Ala Leu Gln Glu Leu Leu Ile Gln Gln Trp Ile Gln Phe Met
        110                 115                 120

atg agc cga cga cga cta cta gcg tgc ctt tgt aag cac aag aaa gtg      974
Met Ser Arg Arg Arg Leu Leu Ala Cys Leu Cys Lys His Lys Lys Val
    125                 130                 135

agt acg aac tta tgt act cat tcg ttt cgg aag aaa cag gta cgt         1019
Ser Thr Asn Leu Cys Thr His Ser Phe Arg Lys Lys Gln Val Arg
140                 145                 150

taatagttaa tagcgtactt ctttttcttg ctttcgtggt attcttgcta gtcacactag   1079

ccatccttac tgcgctt                                                 1096


<210> SEQ ID NO 12
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 12

Met Met Pro Thr Thr Leu Phe Ala Gly Thr His Ile Thr Met Thr Thr
1               5                   10                  15

Val Tyr His Ile Thr Val Ser Gln Ile Gln Leu Ser Leu Leu Lys Val
            20                  25                  30

Thr Ala Phe Gln His Gln Asn Ser Lys Lys Thr Thr Lys Leu Val Val
        35                  40                  45

Ile Leu Arg Ile Gly Thr Gln Val Leu Lys Thr Met Ser Leu Tyr Met
    50                  55                  60

Ala Ile Ser Pro Lys Phe Thr Thr Ser Leu Ser Leu His Lys Leu Leu
65                  70                  75                  80

Gln Thr Leu Val Leu Lys Met Leu His Ser Ser Ser Leu Thr Ser Leu
                85                  90                  95

Leu Lys Thr His Arg Met Cys Lys Tyr Thr Gln Ser Thr Ala Leu Gln
            100                 105                 110

Glu Leu Leu Ile Gln Gln Trp Ile Gln Phe Met Met Ser Arg Arg Arg
        115                 120                 125

Leu Leu Ala Cys Leu Cys Lys His Lys Lys Val Ser Thr Asn Leu Cys
    130                 135                 140

Thr His Ser Phe Arg Lys Lys Gln Val Arg
145                 150


<210> SEQ ID NO 13
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (36)..(263)
<223> OTHER INFORMATION:

<400> SEQUENCE: 13

tgcctttgta agcacaagaa agtgagtacg aacttatg tac tca ttc gtt tcg        53
                                       Met Tyr Ser Phe Val Ser
                                       1               5

gaa gaa aca ggt acg tta ata gtt aat agc gta ctt ctt ttt ctt gct      101
Glu Glu Thr Gly Thr Leu Ile Val Asn Ser Val Leu Leu Phe Leu Ala
            10                  15                  20
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ttc gtg gta ttc ttg cta gtc aca cta gcc atc ctt act gcg ctt cga      149
Phe Val Val Phe Leu Leu Val Thr Leu Ala Ile Leu Thr Ala Leu Arg
        25                  30                  35

ttg tgt gcg tac tgc tgc aat att gtt aac gtg agt tta gta aaa cca      197
Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn Val Ser Leu Val Lys Pro
    40                  45                  50

acg gtt tac gtc tac tcg cgt gtt aaa aat ctg aac tct tct gaa gga      245
Thr Val Tyr Val Tyr Ser Arg Val Lys Asn Leu Asn Ser Ser Glu Gly
55                  60                  65                  70

gtt cct gat ctt ctg gtc taaacgaact aactattatt attattctgt            293
Val Pro Asp Leu Leu Val
                75

ttggaacttt aacattgctt atcatggcag acaacggta                          332


<210> SEQ ID NO 14
<211> LENGTH: 76
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 14

Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu Ile Val Asn Ser
1               5                   10                  15

Val Leu Leu Phe Leu Ala Phe Val Val Phe Leu Leu Val Thr Leu Ala
            20                  25                  30

Ile Leu Thr Ala Leu Arg Leu Cys Ala Tyr Cys Cys Asn Ile Val Asn
        35                  40                  45

Val Ser Leu Val Lys Pro Thr Val Tyr Val Tyr Ser Arg Val Lys Asn
    50                  55                  60

Leu Asn Ser Ser Glu Gly Val Pro Asp Leu Leu Val
65                  70                  75


<210> SEQ ID NO 15
<211> LENGTH: 332
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 15

tgcctttgta agcacaagaa agtgagtacg aacttatgta ctcattcgtt tcggaagaaa     60

caggtacgtt aatagttaat agcgtacttc tttttcttgc tttcgtggta ttcttgctag    120

tcacactagc catccttact gcgcttcgat tgtgtgcgta ctgctgcaat attgttaacg    180

tgagtttagt aaaaccaacg gtttacgtct actcgcgtgt taaaaatctg aactcttctg    240

aaggagttcc tgatcttctg gtctaaacga actaactatt attattattc tgtttggaac    300

tttaacattg cttatcatgg cagacaacgg ta                                 332


<210> SEQ ID NO 16
<211> LENGTH: 708
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (41)..(703)
<223> OTHER INFORMATION:

<400> SEQUENCE: 16

tattattatt attctgtttg gaactttaac attgcttatc atg gca gac aac ggt       55
                                            Met Ala Asp Asn Gly
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                                                 1               5

act att acc gtt gag gag ctt aaa caa ctc ctg gaa caa tgg aac cta      103
Thr Ile Thr Val Glu Glu Leu Lys Gln Leu Leu Glu Gln Trp Asn Leu
                10                  15                  20

gta ata ggt ttc cta ttc cta gcc tgg att atg tta cta caa ttt gcc      151
Val Ile Gly Phe Leu Phe Leu Ala Trp Ile Met Leu Leu Gln Phe Ala
            25                  30                  35

tat tct aat cgg aac agg ttt ttg tac ata ata aag ctt gtt ttc ctc      199
Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile Lys Leu Val Phe Leu
        40                  45                  50

tgg ctc ttg tgg cca gta aca ctt gct tgt ttt gtg ctt gct gct gtc      247
Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe Val Leu Ala Ala Val
    55                  60                  65

tac aga att aat tgg gtg act ggc ggg att gcg att gca atg gct tgt      295
Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala Ile Ala Met Ala Cys
70                  75                  80                  85

att gta ggc ttg atg tgg ctt agc tac ttc gtt gct tcc ttc agg ctg      343
Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val Ala Ser Phe Arg Leu
                90                  95                  100

ttt gct cgt acc cgc tca atg tgg tca ttc aac cca gaa aca aac att      391
Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn Pro Glu Thr Asn Ile
            105                 110                 115

ctt ctc aat gtg cct ctc cgg ggg aca att gtg acc aga ccg ctc atg      439
Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val Thr Arg Pro Leu Met
        120                 125                 130

gaa agt gaa ctt gtc att ggt gct gtg atc att cgt ggt cac ttg cga      487
Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile Arg Gly His Leu Arg
    135                 140                 145

atg gcc gga cac tcc cta ggg cgc tgt gac att aag gac ctg cca aaa      535
Met Ala Gly His Ser Leu Gly Arg Cys Asp Ile Lys Asp Leu Pro Lys
150                 155                 160                 165

gag atc act gtg gct aca tca cga acg ctt tct tat tac aaa tta gga      583
Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser Tyr Tyr Lys Leu Gly
                170                 175                 180

gcg tcg cag cgt gta ggc act gat tca ggt ttt gct gca tac aac cgc      631
Ala Ser Gln Arg Val Gly Thr Asp Ser Gly Phe Ala Ala Tyr Asn Arg
            185                 190                 195

tac cgt att gga aac tat aaa tta aat aca gac cac gcc ggt agc aac      679
Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp His Ala Gly Ser Asn
        200                 205                 210

gac aat att gct ttg cta gta cag taagt                               708
Asp Asn Ile Ala Leu Leu Val Gln
    215                 220


<210> SEQ ID NO 17
<211> LENGTH: 221
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 17

Met Ala Asp Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Gln Leu Leu
1               5                   10                  15

Glu Gln Trp Asn Leu Val Ile Gly Phe Leu Phe Leu Ala Trp Ile Met
            20                  25                  30

Leu Leu Gln Phe Ala Tyr Ser Asn Arg Asn Arg Phe Leu Tyr Ile Ile
        35                  40                  45

Lys Leu Val Phe Leu Trp Leu Leu Trp Pro Val Thr Leu Ala Cys Phe
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    50                  55                  60

Val Leu Ala Ala Val Tyr Arg Ile Asn Trp Val Thr Gly Gly Ile Ala
65                  70                  75                  80

Ile Ala Met Ala Cys Ile Val Gly Leu Met Trp Leu Ser Tyr Phe Val
                85                  90                  95

Ala Ser Phe Arg Leu Phe Ala Arg Thr Arg Ser Met Trp Ser Phe Asn
            100                 105                 110

Pro Glu Thr Asn Ile Leu Leu Asn Val Pro Leu Arg Gly Thr Ile Val
        115                 120                 125

Thr Arg Pro Leu Met Glu Ser Glu Leu Val Ile Gly Ala Val Ile Ile
    130                 135                 140

Arg Gly His Leu Arg Met Ala Gly His Ser Leu Gly Arg Cys Asp Ile
145                 150                 155                 160

Lys Asp Leu Pro Lys Glu Ile Thr Val Ala Thr Ser Arg Thr Leu Ser
                165                 170                 175

Tyr Tyr Lys Leu Gly Ala Ser Gln Arg Val Gly Thr Asp Ser Gly Phe
            180                 185                 190

Ala Ala Tyr Asn Arg Tyr Arg Ile Gly Asn Tyr Lys Leu Asn Thr Asp
        195                 200                 205

His Ala Gly Ser Asn Asp Asn Ile Ala Leu Leu Val Gln
    210                 215                 220
```

<210> SEQ ID NO 18
<211> LENGTH: 769
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 18

```
cctgatcttc tggtctaaac gaactaacta ttattattat tctgtttgga actttaacat     60

tgcttatcat ggcagacaac ggtactatta ccgttgagga gcttaaacaa ctcctggaac    120

aatggaacct agtaataggt ttcctattcc tagcctggat tatgttacta caatttgcct    180

attctaatcg gaacaggttt ttgtacataa taaagcttgt tttcctctgg ctcttgtggc    240

cagtaacact tgcttgtttt gtgcttgctg ctgtctacag aattaattgg gtgactggcg    300

ggattgcgat tgcaatggct tgtattgtag gcttgatgtg gcttagctac ttcgttgctt    360

ccttcaggct gtttgctcgt acccgctcaa tgtggtcatt caacccagaa acaaacattc    420

ttctcaatgt gcctctccgg gggacaattg tgaccagacc gctcatggaa agtgaacttg    480

tcattggtgc tgtgatcatt cgtggtcact tgcgaatggc cggacactcc ctagggcgct    540

gtgacattaa ggacctgcca aaagagatca ctgtggctac atcacgaacg ctttcttatt    600

acaaattagg agcgtcgcag cgtgtaggca ctgattcagg ttttgctgca tacaaccgct    660

accgtattgg aaactataaa ttaaatacag accacgccgg tagcaacgac aatattgctt    720

tgctagtaca gtaagtgaca acagatgttt catcttgttg acttccagg                769
```

<210> SEQ ID NO 19
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 19

```
taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60
```

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ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc 120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat 180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt 240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct 300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag 360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat 420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg 480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac 540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg 600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct 660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat 720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt 780

ctaaacgaac atgaaacttc tcattgtttt gacttgtatt tctctatgca gttgcatatg 840

cactgtagta cagcgctgtg catctaataa acctcatgtg cttgaagatc cttgtaaggt 900

acaacactag gggtaatact tatagcactg cttggctttg tgctctagga aaggttttac 960

cttttcatag atggcacact atggttcaaa catgcacacc taatgttact atcaactgtc 1020

aagatccagc tggtggtgcg cttatagcta ggtgttggta ccttcatgaa ggtcaccaaa 1080

ctgctgcatt tagagacgta cttgttgttt taaataaacg aacaaattaa aatgtctgat 1140

aatggacccc aatcaaacca acgtagtgcc ccccgcatta catttggtgg acccacagat 1200

tcaactgaca ataaccagaa tggaggacgc a 1231

<210> SEQ ID NO 20
<211> LENGTH: 1242
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 20

gcatacaacc gctaccgtat tggaaactat aaattaaata cagaccacgc cggtagcaac 60

gacaatattg ctttgctagt acagtaagtg acaacagatg tttcatcttg ttgacttcca 120

ggttacaata gcagagatat tgattatcat tatgaggact ttcaggattg ctatttggaa 180

tcttgacgtt ataataagtt caatagtgag acagttattt aagcctctaa ctaagaagaa 240

ttattcggag ttagatgatg aagaacctat ggagttagat tatccataaa acgaacatga 300

aaattattct cttcctgaca ttgattgtat ttacatcttg cgagctatat cactatcagg 360

agtgtgttag aggtacgact gtactactaa aagaaccttg cccatcagga acatacgagg 420

gcaattcacc atttcaccct cttgctgaca ataaatttgc actaacttgc actagcacac 480

actttgcttt tgcttgtgct gacggtactc gacataccta tcagctgcgt gcaagatcag 540

tttcaccaaa acttttcatc agacaagagg aggttcaaca agagctctac tcgccacttt 600

ttctcattgt tgctgctcta gtatttttaa tactttgctt caccattaag agaaagacag 660

aatgaatgag ctcactttaa ttgacttcta tttgtgcttt ttagcctttc tgctattcct 720

tgttttaata atgcttatta tattttggtt ttcactcgaa atccaggatc tagaagaacc 780

ttgtaccaaa gtctaaacga acatgaaact tctcattgtt ttgacttgta tttctctatg 840

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cagttgcata tgcactgtag tacagcgctg tgcatctaat aaacctcatg tgcttgaaga    900

tccttgtaag gtacaacact aggggtaata cttatagcac tgcttggctt tgtgctctag    960

gaaaggtttt acctttтcat agatggcaca ctatggttca aacatgcaca cctaatgtta   1020

ctatcaactg tcaagatcca gctggtggtg cgcttatagc taggtgttgg taccttcatg   1080

aaggtcacca aactgctgca tttagagacg tacttgttgt tttaaataaa cgaacgaatt   1140

aaaatgtctg ataatggacc ccaatcaaac caacgtagtg ccccccgcat tacatttggt   1200

ggacccacag attcaactga caataaccag aatggaggac gc                      1242


<210> SEQ ID NO 21
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (86)..(274)
<223> OTHER INFORMATION:

<400> SEQUENCE: 21

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacag atg ttt cat ctt gtt gac ttc cag gtt      112
                            Met Phe His Leu Val Asp Phe Gln Val
                            1               5

aca ata gca gag ata ttg att atc att atg agg act ttc agg att gct      160
Thr Ile Ala Glu Ile Leu Ile Ile Ile Met Arg Thr Phe Arg Ile Ala
10                  15                  20                  25

att tgg aat ctt gac gtt ata ata agt tca ata gtg aga caa tta ttt      208
Ile Trp Asn Leu Asp Val Ile Ile Ser Ser Ile Val Arg Gln Leu Phe
                30                  35                  40

aag cct cta act aag aag aat tat tcg gag tta gat gat gaa gaa cct      256
Lys Pro Leu Thr Lys Lys Asn Tyr Ser Glu Leu Asp Asp Glu Glu Pro
            45                  50                  55

atg gag tta gat tat cca taaaacgaac atgaaaatta ttctcttcct             304
Met Glu Leu Asp Tyr Pro
        60

gacattgatt gtatttacat cttgcgagct atatcactat caggagtgtg ttagaggtac    364

gactgtacta ctaaaagaac cttgcccatc aggaacatac gagggcaatt caccatttca    424

ccctcttgct gacaataaat ttgcactaac ttgcactagc acacactttg cttttgcttg    484

tgctgacggt actcgacata cctatcagct gcgtgcaaga tcagtttcac caaaactttt    544

catcagacaa gaggaggttc aacaagagct ctactcgcca ctttttctca ttgttgctgc    604

tctagtattt ttaatacttt gcttcaccat taagagaaag acagaatgaa tgagctcact    664

ttaattgact tctatttgtg ctttttagcc tttctgctat tccttgtttt aataatgctt    724

attatatttt ggttttcact cgaaatccag gatctagaag aaccttgtac caaagtctaa    784

acgaacatga aacttctcat tgttttgact tgtatttctc tatgcagttg catatgcact    844

gtagtacagc gctgtgcatc taataaacct catgtgcttg aagatccttg taaggtacaa    904

cactaggggt aatacttata gcactgcttg gctttgtgct ctaggaaagg ttttaccttt    964

tcatagatgg cacactatgg ttcaaacatg cacacctaat gttactatca actgtcaaga   1024

tccagctggt ggtgcgctta tagctaggtg ttggtacctt catgaaggtc accaaactgc   1084

tgcatttaga gacgtacttg ttgttttaaa taaacgaaca aattaaaatg tctgataatg   1144
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gaccccaatc aaaccaacgt agtgcccccc gcattacatt tggtggaccc acagattcaa   1204

ctgacaataa ccagaatgga ggacgca                                      1231


<210> SEQ ID NO 22
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 22

Met Phe His Leu Val Asp Phe Gln Val Thr Ile Ala Glu Ile Leu Ile
1               5                  10                  15

Ile Ile Met Arg Thr Phe Arg Ile Ala Ile Trp Asn Leu Asp Val Ile
            20                  25                  30

Ile Ser Ser Ile Val Arg Gln Leu Phe Lys Pro Leu Thr Lys Lys Asn
        35                  40                  45

Tyr Ser Glu Leu Asp Asp Glu Glu Pro Met Glu Leu Asp Tyr Pro
    50                  55                  60


<210> SEQ ID NO 23
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (285)..(650)
<223> OTHER INFORMATION:

<400> SEQUENCE: 23

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaac atg aaa att att      296
                                                Met Lys Ile Ile
                                                1

ctc ttc ctg aca ttg att gta ttt aca tct tgc gag cta tat cac tat      344
Leu Phe Leu Thr Leu Ile Val Phe Thr Ser Cys Glu Leu Tyr His Tyr
5                   10                  15                  20

cag gag tgt gtt aga ggt acg act gta cta cta aaa gaa cct tgc cca      392
Gln Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys Glu Pro Cys Pro
                25                  30                  35

tca gga aca tac gag ggc aat tca cca ttt cac cct ctt gct gac aat      440
Ser Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro Leu Ala Asp Asn
            40                  45                  50

aaa ttt gca cta act tgc act agc aca cac ttt gct ttt gct tgt gct      488
Lys Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala Phe Ala Cys Ala
        55                  60                  65

gac ggt act cga cat acc tat cag ctg cgt gca aga tca gtt tca cca      536
Asp Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg Ser Val Ser Pro
    70                  75                  80

aaa ctt ttc atc aga caa gag gag gtt caa caa gag ctc tac tcg cca      584
Lys Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu Leu Tyr Ser Pro
85                  90                  95                  100

ctt ttt ctc att gtt gct gct cta gta ttt tta ata ctt tgc ttc acc      632
Leu Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile Leu Cys Phe Thr
                105                 110                 115
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att aag aga aag aca gaa tgaatgagct cactttaatt gacttctatt            680
Ile Lys Arg Lys Thr Glu
            120

tgtgcttttt agcctttctg ctattccttg ttttaataat gcttattata ttttggtttt   740

cactcgaaat ccaggatcta gaagaacctt gtaccaaagt ctaaacgaac atgaaacttc   800

tcattgtttt gacttgtatt tctctatgca gttgcatatg cactgtagta cagcgctgtg   860

catctaataa acctcatgtg cttgaagatc cttgtaaggt acaacactag gggtaatact   920

tatagcactg cttggctttg tgctctagga aaggttttac cttttcatag atggcacact   980

atggttcaaa catgcacacc taatgttact atcaactgtc aagatccagc tggtggtgcg  1040

cttatagcta ggtgttggta ccttcatgaa ggtcaccaaa ctgctgcatt tagagacgta  1100

cttgttgttt taaataaacg aacaaattaa aatgtctgat aatggacccc aatcaaacca  1160

acgtagtgcc ccccgcatta catttggtgg acccacagat tcaactgaca ataaccagaa  1220

tggaggacgc a                                                       1231


<210> SEQ ID NO 24
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 24

Met Lys Ile Ile Leu Phe Leu Thr Leu Ile Val Phe Thr Ser Cys Glu
1               5                   10                  15

Leu Tyr His Tyr Gln Glu Cys Val Arg Gly Thr Thr Val Leu Leu Lys
            20                  25                  30

Glu Pro Cys Pro Ser Gly Thr Tyr Glu Gly Asn Ser Pro Phe His Pro
        35                  40                  45

Leu Ala Asp Asn Lys Phe Ala Leu Thr Cys Thr Ser Thr His Phe Ala
    50                  55                  60

Phe Ala Cys Ala Asp Gly Thr Arg His Thr Tyr Gln Leu Arg Ala Arg
65                  70                  75                  80

Ser Val Ser Pro Lys Leu Phe Ile Arg Gln Glu Glu Val Gln Gln Glu
                85                  90                  95

Leu Tyr Ser Pro Leu Phe Leu Ile Val Ala Ala Leu Val Phe Leu Ile
            100                 105                 110

Leu Cys Phe Thr Ile Lys Arg Lys Thr Glu
        115                 120


<210> SEQ ID NO 25
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (650)..(781)
<223> OTHER INFORMATION:

<400> SEQUENCE: 25

taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240
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agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacaga atg aat gag    658
                                                    Met Asn Glu
                                                    1

ctc act tta att gac ttc tat ttg tgc ttt tta gcc ttt ctg cta ttc      706
Leu Thr Leu Ile Asp Phe Tyr Leu Cys Phe Leu Ala Phe Leu Leu Phe
    5                  10                  15

ctt gtt tta ata atg ctt att ata ttt tgg ttt tca ctc gaa atc cag      754
Leu Val Leu Ile Met Leu Ile Ile Phe Trp Phe Ser Leu Glu Ile Gln
20                  25                  30                  35

gat cta gaa gaa cct tgt acc aaa gtc taaacgaaca tgaaacttct            801
Asp Leu Glu Glu Pro Cys Thr Lys Val
                40

cattgttttg acttgtattt ctctatgcag ttgcatatgc actgtagtac agcgctgtgc    861

atctaataaa cctcatgtgc ttgaagatcc ttgtaaggta caacactagg ggtaatactt    921

atagcactgc ttggctttgt gctctaggaa aggttttacc ttttcataga tggcacacta    981

tggttcaaac atgcacacct aatgttacta tcaactgtca agatccagct ggtggtgcgc   1041

ttatagctag gtgttggtac cttcatgaag gtcaccaaac tgctgcattt agagacgtac   1101

ttgttgtttt aaataaacga acaaattaaa atgtctgata atggacccca atcaaaccaa   1161

cgtagtgccc cccgcattac atttggtgga cccacagatt caactgacaa taaccagaat   1221

ggaggacgca                                                          1231
```

<210> SEQ ID NO 26
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 26

```
Met Asn Glu Leu Thr Leu Ile Asp Phe Tyr Leu Cys Phe Leu Ala Phe
1               5                  10                  15

Leu Leu Phe Leu Val Leu Ile Met Leu Ile Ile Phe Trp Phe Ser Leu
            20                  25                  30

Glu Ile Gln Asp Leu Glu Glu Pro Cys Thr Lys Val
        35                  40
```

<210> SEQ ID NO 27
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (791)..(907)
<223> OTHER INFORMATION:

<400> SEQUENCE: 27

```
taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120
```

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agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct    660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat    720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt    780

ctaaacgaac atg aaa ctt ctc att gtt ttg act tgt att tct cta tgc       829
           Met Lys Leu Leu Ile Val Leu Thr Cys Ile Ser Leu Cys
           1               5                   10

agt tgc ata tgc act gta gta cag cgc tgt gca tct aat aaa cct cat      877
Ser Cys Ile Cys Thr Val Val Gln Arg Cys Ala Ser Asn Lys Pro His
    15                  20                  25

gtg ctt gaa gat cct tgt aag gta caa cac taggggtaat acttatagca        927
Val Leu Glu Asp Pro Cys Lys Val Gln His
30                  35

ctgcttggct ttgtgctcta ggaaaggttt tacctttca tagatggcac actatggttc     987

aaacatgcac acctaatgtt actatcaact gtcaagatcc agctggtggt gcgcttatag   1047

ctaggtgttg gtaccttcat gaaggtcacc aaactgctgc atttagagac gtacttgttg   1107

ttttaaataa acgaacaaat taaaatgtct gataatggac cccaatcaaa ccaacgtagt   1167

gccccccgca ttacatttgg tggacccaca gattcaactg acaataacca gaatggagga   1227

cgca                                                                1231
```

<210> SEQ ID NO 28
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 28

```
Met Lys Leu Leu Ile Val Leu Thr Cys Ile Ser Leu Cys Ser Cys Ile
1               5                   10                  15

Cys Thr Val Val Gln Arg Cys Ala Ser Asn Lys Pro His Val Leu Glu
            20                  25                  30

Asp Pro Cys Lys Val Gln His
        35
```

<210> SEQ ID NO 29
<211> LENGTH: 1231
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (876)..(1127)
<223> OTHER INFORMATION:

<400> SEQUENCE: 29

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taccgtattg gaaactataa attaaataca gaccacgccg gtagcaacga caatattgct     60

ttgctagtac agtaagtgac aacagatgtt tcatcttgtt gacttccagg ttacaatagc    120

agagatattg attatcatta tgaggacttt caggattgct atttggaatc ttgacgttat    180

aataagttca atagtgagac aattatttaa gcctctaact aagaagaatt attcggagtt    240

agatgatgaa gaacctatgg agttagatta tccataaaac gaacatgaaa attattctct    300

tcctgacatt gattgtattt acatcttgcg agctatatca ctatcaggag tgtgttagag    360

gtacgactgt actactaaaa gaaccttgcc catcaggaac atacgagggc aattcaccat    420

ttcaccctct tgctgacaat aaatttgcac taacttgcac tagcacacac tttgcttttg    480

cttgtgctga cggtactcga catacctatc agctgcgtgc aagatcagtt tcaccaaaac    540

ttttcatcag acaagaggag gttcaacaag agctctactc gccacttttt ctcattgttg    600

ctgctctagt atttttaata ctttgcttca ccattaagag aaagacagaa tgaatgagct    660

cactttaatt gacttctatt tgtgcttttt agcctttctg ctattccttg ttttaataat    720

gcttattata ttttggtttt cactcgaaat ccaggatcta gaagaacctt gtaccaaagt    780

ctaaacgaac atgaaacttc tcattgtttt gacttgtatt tctctatgca gttgcatatg    840

cactgtagta cagcgctgtg catctaataa acctc atg tgc ttg aag atc ctt       893
                                      Met Cys Leu Lys Ile Leu
                                      1               5

gta agg tac aac act agg ggt aat act tat agc act gct tgg ctt tgt      941
Val Arg Tyr Asn Thr Arg Gly Asn Thr Tyr Ser Thr Ala Trp Leu Cys
            10                  15                  20

gct cta gga aag gtt tta cct ttt cat aga tgg cac act atg gtt caa      989
Ala Leu Gly Lys Val Leu Pro Phe His Arg Trp His Thr Met Val Gln
        25                  30                  35

aca tgc aca cct aat gtt act atc aac tgt caa gat cca gct ggt ggt     1037
Thr Cys Thr Pro Asn Val Thr Ile Asn Cys Gln Asp Pro Ala Gly Gly
    40                  45                  50

gcg ctt ata gct agg tgt tgg tac ctt cat gaa ggt cac caa act gct     1085
Ala Leu Ile Ala Arg Cys Trp Tyr Leu His Glu Gly His Gln Thr Ala
55                  60                  65                  70

gca ttt aga gac gta ctt gtt gtt tta aat aaa cga aca aat              1127
Ala Phe Arg Asp Val Leu Val Val Leu Asn Lys Arg Thr Asn
                75                  80

taaaatgtct gataatggac cccaatcaaa ccaacgtagt gccccccgca ttacatttgg   1187

tggacccaca gattcaactg acaataacca gaatggagga cgca                   1231


<210> SEQ ID NO 30
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 30

Met Cys Leu Lys Ile Leu Val Arg Tyr Asn Thr Arg Gly Asn Thr Tyr
1               5                   10                  15

Ser Thr Ala Trp Leu Cys Ala Leu Gly Lys Val Leu Pro Phe His Arg
            20                  25                  30

Trp His Thr Met Val Gln Thr Cys Thr Pro Asn Val Thr Ile Asn Cys
        35                  40                  45

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tacgcgtata tgctaactta ggtgagcgtg tacgccaatc attattaaag actgtacaat 13680

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ccccagcaca tgtatctaca ataggtgtct gcacaatgac tgacattgcc aagaaaccta 19620

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ggtgtaagga tggacatgtt gaaaccttct acccaaaact acaagcaagt caagcgtggc 20340

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agtatactca actgtgtcaa tacttaaata cacttacttt agctgtaccc tacaacatga 20520

gagttattca ctttggtgct ggctctgata aaggagttgc accaggtaca gctgtgctca 20580

gacaatggtt gccaactggc acactacttg tcgattcaga tcttaatgac ttcgtctccg 20640

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aagaagggtt tttcacttat ctgtgtggat ttataaagca aaaactagcc ctgggtggtt 20820

ctatagctgt aaagataaca gagcattctt ggaatgctga cctttacaag cttatgggcc 20880

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ctaactacat tttctggagg aacacaaatc ctatccagtt gtcttcctat tcactctttg 21060

acatgagcaa atttcctctt aaattaagag gaactgctgt aatgtctctt aaggagaatc 21120

aaatcaatga tatgatttat tctcttctgg aaaaaggtag gcttatcatt agagaaaaca 21180

acagagttgt ggtttcaagt gatattcttg ttaacaacta a 21221

<210> SEQ ID NO 32
<211> LENGTH: 297
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 32

atggacccca atcaaaccaa cgtagtgccc cccgcattac atttggtgga cccacagatt 60

caactgacaa taaccagaat ggaggacgca atggggcaag gccaaaacag cgccgacccc 120

aaggtttacc caataatact gcgtcttggt tcacagctct cactcagcat ggcaaggagg 180

aacttagatt ccctcgaggc cagggcgttc caatcaacac caatagtggt ccagatgacc 240

aaattggcta ctaccgaaga gctacccgac gagttcgtgg tggtgacggc aaaatga 297

<210> SEQ ID NO 33
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 33

Met Asp Pro Asn Gln Thr Asn Val Val Pro Pro Ala Leu His Leu Val
1 5 10 15

Asp Pro Gln Ile Gln Leu Thr Ile Thr Arg Met Glu Asp Ala Met Gly
20 25 30

Gln Gly Gln Asn Ser Ala Asp Pro Lys Val Tyr Pro Ile Ile Leu Arg
35 40 45

Leu Gly Ser Gln Leu Ser Leu Ser Met Ala Arg Arg Asn Leu Asp Ser
50 55 60

Leu Glu Ala Arg Ala Phe Gln Ser Thr Pro Ile Val Val Gln Met Thr

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65                  70                  75                  80

Lys Leu Ala Thr Thr Glu Glu Leu Pro Asp Glu Phe Val Val Val Thr
                85                  90                  95

Ala Lys


<210> SEQ ID NO 34
<211> LENGTH: 213
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 34

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agggaagcag aggcggcagt caagcctctt ctcgctcctc atcacgtagt cgcggtaatt    120

caagaaattc aactcctggc agcagtaggg gaaattctcc tgctcgaatg gctagcggag    180

gtggtgaaac tgccctcgcg ctattgctgc tag                                 213


<210> SEQ ID NO 35
<211> LENGTH: 70
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 35

Met Leu Pro Pro Cys Tyr Asn Phe Leu Lys Glu Gln His Cys Gln Lys
1               5                   10                  15

Ala Ser Thr Gln Arg Glu Ala Glu Ala Ala Val Lys Pro Leu Leu Ala
            20                  25                  30

Pro His His Val Val Ala Val Ile Gln Glu Ile Gln Leu Leu Ala Ala
        35                  40                  45

Val Gly Glu Ile Leu Leu Leu Glu Trp Leu Ala Glu Val Val Lys Leu
    50                  55                  60

Pro Ser Arg Tyr Cys Cys
65                  70


<210> SEQ ID NO 36
<211> LENGTH: 1377
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (67)..(1335)
<223> OTHER INFORMATION:

<400> SEQUENCE: 36

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attaaa atg tct gat aat gga ccc caa tca aac caa cgt agt gcc ccc       108
       Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro
       1               5                   10

cgc att aca ttt ggt gga ccc aca gat tca act gac aat aac cag aat      156
Arg Ile Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn Asn Gln Asn
15                  20                  25                  30

gga gga cgc aat ggg gca agg cca aaa cag cgc cga ccc caa ggt tta      204
Gly Gly Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro Gln Gly Leu
                35                  40                  45

ccc aat aat act gcg tct tgg ttc aca gct ctc act cag cat ggc aag      252
Pro Asn Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys
            50                  55                  60
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gag gaa ctt aga ttc cct cga ggc cag ggc gtt cca atc aac acc aat      300
Glu Glu Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn
        65                  70                  75

agt ggt cca gat gac caa att ggc tac tac cga aga gct acc cga cga      348
Ser Gly Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg
    80                  85                  90

gtt cgt ggt ggt gac ggc aaa atg aaa gag ctc agc ccc aga tgg tac      396
Val Arg Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro Arg Trp Tyr
95                  100                 105                 110

ttc tat tac cta gga act ggc cca gaa gct tca ctt ccc tac ggc gct      444
Phe Tyr Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro Tyr Gly Ala
                115                 120                 125

aac aaa gaa ggc atc gta tgg gtt gca act gag gga gcc ttg aat aca      492
Asn Lys Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr
            130                 135                 140

ccc aaa gac cac att ggc acc cgc aat cct aat aac aat gct gcc acc      540
Pro Lys Asp His Ile Gly Thr Arg Asn Pro Asn Asn Asn Ala Ala Thr
        145                 150                 155

gtg cta caa ctt cct caa gga aca aca ttg cca aaa ggc ttc tac gca      588
Val Leu Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala
    160                 165                 170

gag gga agc aga ggc ggc agt caa gcc tct tct cgc tcc tca tca cgt      636
Glu Gly Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg
175                 180                 185                 190

agt cgc ggt aat tca aga aat tca act cct ggc agc agt agg gga aat      684
Ser Arg Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn
                195                 200                 205

tct cct gct cga atg gct agc gga ggt ggt gaa act gcc ctc gcg cta      732
Ser Pro Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu
            210                 215                 220

ttg ctg cta gac aga ttg aac cag ctt gag agc aaa gtt tct ggt aaa      780
Leu Leu Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys
        225                 230                 235

ggc caa caa caa caa ggc caa act gtc act aag aaa tct gct gct gag      828
Gly Gln Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu
    240                 245                 250

gca tct aaa aag cct cgc caa aaa cgt act gcc aca aaa cag tac aac      876
Ala Ser Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn
255                 260                 265                 270

gtc act caa gca ttt ggg aga cgt ggt cca gaa caa acc caa gga aat      924
Val Thr Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn
                275                 280                 285

ttc ggg gac caa gac cta atc aga caa gga act gat tac aaa cat tgg      972
Phe Gly Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp
            290                 295                 300

ccg caa att gca caa ttt gct cca agt gcc tct gca ttc ttt gga atg     1020
Pro Gln Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met
        305                 310                 315

tca cgc att ggc atg gaa gtc aca cct tcg gga aca tgg ctg act tat     1068
Ser Arg Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr
    320                 325                 330

cat gga gcc att aaa ttg gat gac aaa gat cca caa ttc aaa gac aac     1116
His Gly Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn
335                 340                 345                 350

gtc ata ctg ctg aac aag cac att gac gca tac aaa aca ttc cca cca     1164
Val Ile Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro
                355                 360                 365
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aca gag cct aaa aag gac aaa aag aaa aag act gat gaa gct cag cct     1212
Thr Glu Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro
            370                 375                 380

ttg ccg cag aga caa aag aag cag ccc act gtg act ctt ctt cct gcg     1260
Leu Pro Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala
        385                 390                 395

gct gac atg gat gat ttc tcc aga caa ctt caa aat tcc atg agt gga     1308
Ala Asp Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly
    400                 405                 410

gct tct gct gat tca act cag gca taa acactcatga tgaccacaca          1355
Ala Ser Ala Asp Ser Thr Gln Ala
415                 420

aggcagatgg gctatgtaaa cg                                           1377


<210> SEQ ID NO 37
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 37

Met Ser Asp Asn Gly Pro Gln Ser Asn Gln Arg Ser Ala Pro Arg Ile
1               5                   10                  15

Thr Phe Gly Gly Pro Thr Asp Ser Thr Asp Asn Asn Gln Asn Gly Gly
            20                  25                  30

Arg Asn Gly Ala Arg Pro Lys Gln Arg Arg Pro Gln Gly Leu Pro Asn
        35                  40                  45

Asn Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Glu
    50                  55                  60

Leu Arg Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Gly
65                  70                  75                  80

Pro Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Val Arg
                85                  90                  95

Gly Gly Asp Gly Lys Met Lys Glu Leu Ser Pro Arg Trp Tyr Phe Tyr
            100                 105                 110

Tyr Leu Gly Thr Gly Pro Glu Ala Ser Leu Pro Tyr Gly Ala Asn Lys
        115                 120                 125

Glu Gly Ile Val Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys
    130                 135                 140

Asp His Ile Gly Thr Arg Asn Pro Asn Asn Asn Ala Ala Thr Val Leu
145                 150                 155                 160

Gln Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly
                165                 170                 175

Ser Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg
            180                 185                 190

Gly Asn Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Asn Ser Pro
        195                 200                 205

Ala Arg Met Ala Ser Gly Gly Gly Glu Thr Ala Leu Ala Leu Leu Leu
    210                 215                 220

Leu Asp Arg Leu Asn Gln Leu Glu Ser Lys Val Ser Gly Lys Gly Gln
225                 230                 235                 240

Gln Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser
                245                 250                 255

Lys Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Gln Tyr Asn Val Thr
            260                 265                 270
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Gln Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly
        275                 280                 285

Asp Gln Asp Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln
    290                 295                 300

Ile Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg
305                 310                 315                 320

Ile Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr His Gly
                325                 330                 335

Ala Ile Lys Leu Asp Asp Lys Asp Pro Gln Phe Lys Asp Asn Val Ile
            340                 345                 350

Leu Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu
        355                 360                 365

Pro Lys Lys Asp Lys Lys Lys Lys Thr Asp Glu Ala Gln Pro Leu Pro
    370                 375                 380

Gln Arg Gln Lys Lys Gln Pro Thr Val Thr Leu Leu Pro Ala Ala Asp
385                 390                 395                 400

Met Asp Asp Phe Ser Arg Gln Leu Gln Asn Ser Met Ser Gly Ala Ser
                405                 410                 415

Ala Asp Ser Thr Gln Ala
            420
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<210> SEQ ID NO 38
<211> LENGTH: 1377
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 38

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atgaaggtca ccaaactgct gcatttagag acgtacttgt tgttttaaat aaacgaacaa     60

attaaaatgt ctgataatgg accccaatca aaccaacgta gtgccccccg cattacattt    120

ggtggaccca cagattcaac tgacaataac cagaatggag gacgcaatgg ggcaaggcca    180

aaacagcgcc gaccccaagg tttacccaat aatactgcgt cttggttcac agctctcact    240

cagcatggca aggaggaact tagattccct cgaggccagg gcgttccaat caacaccaat    300

agtggtccag atgaccaaat tggctactac cgaagagcta cccgacgagt tcgtggtggt    360

gacggcaaaa tgaaagagct cagccccaga tggtacttct attacctagg aactggccca    420

gaagcttcac ttccctacgg cgctaacaaa gaaggcatcg tatgggttgc aactgaggga    480

gccttgaata cacccaaaga ccacattggc acccgcaatc ctaataacaa tgctgccacc    540

gtgctacaac ttcctcaagg aacaacattg ccaaaaggct tctacgcaga gggaagcaga    600

ggcggcagtc aagcctcttc tcgctcctca tcacgtagtc gcggtaattc aagaaattca    660

actcctggca gcagtagggg aaattctcct gctcgaatgg ctagcggagg tggtgaaact    720

gccctcgcgc tattgctgct agacagattg aaccagcttg agagcaaagt ttctggtaaa    780

ggccaacaac aacaaggcca aactgtcact aagaaatctg ctgctgaggc atctaaaaag    840

cctcgccaaa aacgtactgc cacaaaacag tacaacgtca ctcaagcatt tgggagacgt    900

ggtccagaac aaacccaagg aaatttcggg gaccaagacc taatcagaca aggaactgat    960

tacaaacatt ggccgcaaat tgcacaattt gctccaagtg cctctgcatt ctttggaatg   1020

tcacgcattg gcatggaagt cacaccttcg ggaacatggc tgacttatca tggagccatt   1080

aaattggatg acaaagatcc acaattcaaa gacaacgtca tactgctgaa caagcacatt   1140
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gacgcataca aaacattccc accaacagag cctaaaaagg acaaaaagaa aaagactgat 1200

gaagctcagc ctttgccgca gagacaaaag aagcagccca ctgtgactct tcttcctgcg 1260

gctgacatgg atgatttctc cagacaactt caaaattcca tgagtggagc ttctgctgat 1320

tcaactcagg cataaacact catgatgacc acacaaggca gatgggctat gtaaacg 1377

<210> SEQ ID NO 39
<211> LENGTH: 204
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 39

atattaggtt tttacctacc caggaaaagc caaccaacct cgatctcttg tagatctgtt 60

ctctaaacga actttaaaat ctgtgtagct gtcgctcggc tgcatgccta gtgcacctac 120

gcagtataaa caataataaa ttttactgtc gttgacaaga aacgagtaac tcgtccctct 180

tctgcagact gcttacggtt tcgt 204

<210> SEQ ID NO 40
<211> LENGTH: 809
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 40

actcaagcat ttgggagacg tggtccagaa caaacccaag gaaatttcgg ggaccaagac 60

ctaatcagac aaggaactga ttacaaacat tggccgcaaa ttgcacaatt tgctccaagt 120

gcctctgcat tctttggaat gtcacgcatt ggcatggaag tcacaccttc gggaacatgg 180

ctgacttatc atggagccat taaattggat gacaaagatc cacaattcaa agacaacgtc 240

atactgctga acaagcacat tgacgcatac aaaacattcc caccaacaga gcctaaaaag 300

gacaaaaaga aaaagactga tgaagctcag cctttgccgc agagacaaaa gaagcagccc 360

actgtgactc ttcttcctgc ggctgacatg gatgatttct ccagacaact tcaaaattcc 420

atgagtggag cttctgctga ttcaactcag gcataaacac tcatgatgac cacacaaggc 480

agatgggcta tgtaaacgtt ttcgcaattc cgtttacgat acatagtcta ctcttgtgca 540

gaatgaattc tcgtaactaa acagcacaag taggtttagt taactttaat ctcacatagc 600

aatctttaat caatgtgtaa cattagggag gacttgaaag agccaccaca ttttcatcga 660

ggccacgcgg agtacgatcg agggtacagt gaataatgct agggagagct gcctatatgg 720

aagagcccta atgtgtaaaa ttaattttag tagtgctatc cccatgtgat tttaatagct 780

tcttaggaga atgacaaaaa aaaaaaaaa 809

<210> SEQ ID NO 41
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 41

aatgaacaca tagggctgtt caagctgggg cagtacgcct ttttccagct ctactagacc 60

acaagtgcca tttttgaggt gttcacgtgc ctccgatagg gcctcttcca cagagtcccc 120

gaagccacgc actagcacgt ctctaacctg aaggacaggc aaactgagtt ggacgtgtgt 180

tttctcgttg acaccaagaa caaggctctc catcttacct ttcggtcaca cccggacgaa 240

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acctaggtat gctgatgatc gactgcaaca cggacgaaac cgtaagcagt ctgcagaaga 300

gggacgagtt actcgtttct tgtcaacgac agtaaaattt attattgttt atactgcgta 360

ggtgcactag gcatgcagcc gagcgacagc tacacagatt ttaaagttcg tttagagaac 420

agatctacaa gagatcgagg ttggttgg 448

<210> SEQ ID NO 42
<211> LENGTH: 2033
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 42

atacctaggt ttcgtccggg tgtgaccgaa aggtaagatg gagagccttg ttcttggtgt 60

caacgagaaa acacacgtcc aactcagttt gcctgtcctt caggttagag acgtgctagt 120

gcgtggcttc ggggactctg tggaagaggc cctatcggag gcacgtgaac acctcaaaaa 180

tggcacttgt ggtctagtag agctggaaaa aggcgtactg ccccagcttg aacagcccta 240

tgtgttcatt aaacgttctg atgccttaag caccaatcac ggccacaagg tcgttgagct 300

ggttgcagaa atggacggca ttcagtacgg tcgtagcggt ataacactgg gagtactcgt 360

gccacatgtg ggcgaaaccc caattgcata ccgcaatgtt cttcttcgta agaacggtaa 420

taagggagcc ggtggtcata gctatggcat cgatctaaag tcttatgact taggtgacga 480

gcttggcact gatcccattg aagattatga acaaaactgg aacactaagc atggcagtgg 540

tgcactccgt gaactcactc gtgagctcaa tggaggtgca gtcactcgct atgtcgacaa 600

caatttctgt ggcccagatg ggtaccctct tgattgcatc aaagattttc tcgcacgcgc 660

gggcaagtca atgtgcactc tttccgaaca acttgattac atcgagtcga agagaggtgt 720

ctactgctgc cgtgaccatg agcatgaaat tgcctggttc actgagcgct ctgataagag 780

ctacgagcac cagacaccct tcgaaattaa gagtgccaag aaatttgaca ctttcaaagg 840

ggaatgccca aagtttgtgt ttcctcttaa ctcaaaagtc aaagtcattc aaccacgtgt 900

tgaaaagaaa aagactgagg gtttcatggg gcgtatacgc tctgtgtacc ctgttgcatc 960

tccacaggag tgtaacaata tgcacttgtc taccttgatg aaatgtaatc attgcgatga 1020

agtttcatgg cagacgtgcg actttctgaa agccacttgt gaacattgtg gcactgaaaa 1080

tttagttatt gaaggaccta ctacatgtgg gtacctacct actaatgctg tagtgaaaat 1140

gccatgtcct gcctgtcaag acccagagat tggacctgag catagtgttg cagattatca 1200

caaccactca aacattgaaa ctcgactccg caagggaggt aggactagat gttttggagg 1260

ctgtgtgttt gcctatgttg gctgctataa taagcgtgcc tactgggttc ctcgtgctag 1320

tgctgatatt ggctcaggcc atactggcat tactggtgac aatgtggaga ccttgaatga 1380

ggatctcctt gagatactga gtcgtgaacg tgttaacatt aacattgttg gcgattttca 1440

tttgaatgaa gaggttgcca tcattttggc atctttctct gcttctacaa gtgcctttat 1500

tgacactata aagagtcttg attacaagtc tttcaaaacc attgttgagt cctgcggtaa 1560

ctataaagtt accaagggaa agcccgtaaa aggtgcttgg aacattggac aacagagatc 1620

agttttaaca ccactgtgtg gttttccctc acaggctgct ggtgttatca gatcaatttt 1680

tgcgcgcaca cttgatgcag caaaccactc aattcctgat ttgcaaagag cagctgtcac 1740

catacttgat ggtatttctg aacagtcatt acgtcttgtc gacgccatgg tttatacttc 1800

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agacctgctc accaacagtg tcattattat ggcatatgta actggtggtc ttgtacaaca 1860

gacttctcag tggttgtcta atcttttggg cactactgtt gaaaaactca ggcctatctt 1920

tgaatggatt gaggcgaaac ttagtgcagg agttgaattt ctcaaggatg cttgggagat 1980

tctcaaattt ctcattacag gtgtttttga catcgtcaag ggtcaaatac agg 2033

<210> SEQ ID NO 43
<211> LENGTH: 2018
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 43

ggattgaggc gaaacttagt gcaggagttg aatttctcaa ggatgcttgg gagattctca 60

aatttctcat tacaggtgtt tttgacatcg tcaagggtca aatacaggtt gcttcagata 120

acatcaagga ttgtgtaaaa tgcttcattg atgttgttaa caaggcactc gaaatgtgca 180

ttgatcaagt cactatcgct ggcgcaaagt tgcgatcact caacttaggt gaagtcttca 240

tcgctcaaag caagggactt taccgtcagt gtatacgtgg caaggagcag ctgcaactac 300

tcatgcctct taaggcacca aaagaagtaa cctttcttga aggtgattca catgacacag 360

tacttacctc tgaggaggtt gttctcaaga acggtgaact cgaagcactc gagacgcccg 420

ttgatagctt cacaaatgga gctatcgttg gcacaccagt ctgtgtaaat ggcctcatgc 480

tcttagagat taaggacaaa gaacaatact gcgcattgtc tcctggttta ctggctacaa 540

acaatgtctt tcgcttaaaa gggggtgcac caattaaagg tgtaaccttt ggagaagata 600

ctgtttggga agttcaaggt tacaagaatg tgagaatcac atttgagctt gatgaacgtg 660

ttgacaaagt gcttaatgaa aagtgctctg tctacactgt tgaatccggt accgaagtta 720

ctgagtttgc atgtgttgta gcagaggctg ttgtgaagac tttacaacca gtttctgatc 780

tccttaccaa catgggtatt gatcttgatg agtggagtgt agctacattc tacttatttg 840

atgatgctgg tgaagaaaac ttttcatcac gtatgtattg ttccttttac cctccagatg 900

aggaagaaga ggacgatgca gagtgtgagg aagaagaaat tgatgaaacc tgtgaacatg 960

agtacggtac agaggatgat tatcaaggtc tccctctgga atttggtgcc tcagctgaaa 1020

cagttcgagt tgaggaagaa gaagaggaag actggctgga tgatactact gagcaatcag 1080

agattgagcc agaaccagaa cctacacctg aagaaccagt taatcagttt actggttatt 1140

taaaacttac tgacaatgtt gccattaaat gtgttgacat cgttaaggag gcacaaagtg 1200

ctaatcctat ggtgattgta aatgctgcta acatacacct gaaacatggt ggtggtgtag 1260

caggtgcact caacaaggca accaatggtg ccatgcaaaa ggagagtgat gattacatta 1320

agctaaatgg ccctcttaca gtaggagggt cttgtttgct ttctggacat aatcttgcta 1380

agaagtgtct gcatgttgtt ggacctaacc taaatgcagg tgaggacatc cagcttctta 1440

aggcagcata tgaaaatttc aattcacagg acatcttact tgcaccattg ttgtcagcag 1500

gcatatttgg tgctaaacca cttcagtctt tacaagtgtg cgtgcagacg gttcgtacac 1560

aggtttatat tgcagtcaat gacaaagctc tttatgagca ggttgtcatg gattatcttg 1620

ataacctgaa gcctagagtg gaagcaccta aacaagagga gccaccaaac acagaagatt 1680

ccaaaactga ggagaaatct gtcgtacaga agcctgtcga tgtgaagcca aaaattaagg 1740

cctgcattga tgaggttacc acaacactgg aagaaactaa gtttcttacc aataagttac 1800

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tcttgtttgc tgatatcaat ggtaagcttt accatgattc tcagaacatg cttagaggtg 1860

aagatatgtc tttccttgag aaggatgcac cttacatggt aggtgatgtt atcactagtg 1920

gtgatatcac ttgtgttgta ataccctcca aaaaggctgg tggcactact gagatgctct 1980

caagagcttt gaagaaagtg ccagttgatg agtatata 2018

<210> SEQ ID NO 44
<211> LENGTH: 1442
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 44

ttgatgaggt taccacaaca ctggaagaaa ctaagtttct taccaataag ttactcttgt 60

ttgctgatat caatggtaag ctttaccatg attctcagaa catgcttaga ggtgaagata 120

tgtctttcct tgagaaggat gcaccttaca tggtaggtga tgttatcact agtggtgata 180

tcacttgtgt tgtaataccc tccaaaaagg ctggtggcac tactgagatg ctctcaagag 240

ctttgaagaa agtgccagtt gatgagtata taaccacgta ccctggacaa ggatgtgctg 300

gttatacact tgaggaagct aagactgctc ttaagaaatg caaatctgca ttttatgtac 360

taccttcaga agcacctaat gctaaggaag agattctagg aactgtatcc tggaatttga 420

gagaaatgct tgctcatgct gaagagacaa gaaaattaat gcctatatgc atggatgtta 480

gagccataat ggcaaccatc caacgtaagt ataaaggaat taaaattcaa gagggcatcg 540

ttgactatgg tgtccgattc ttcttttata ctagtaaaga gcctgtagct tctattatta 600

cgaagctgaa ctctctaaat gagccgcttg tcacaatgcc aattggttat gtgacacatg 660

gttttaatct tgaagaggct gcgcgctgta tgcgttctct taaagctcct gccgtagtgt 720

cagtatcatc accagatgct gttactacat ataatggata cctcacttcg tcatcaaaga 780

catctgagga gcactttgta gaaacagttt ctttggctgg ctcttacaga gattggtcct 840

attcaggaca gcgtacagag ttaggtgttg aatttcttaa gcgtggtgac aaaattgtgt 900

accacactct ggagagcccc gtcgagtttc atcttgacgg tgaggttctt tcacttgaca 960

aactaaagag tctcttatcc ctgcgggagg ttaagactat aaaagtgttc acaactgtgg 1020

acaacactaa tctccacaca cagcttgtgg atatgtctat gacatatgga cagcagtttg 1080

gtccaacata cttggatggt gctgatgtta caaaaattaa acctcatgta aatcatgagg 1140

gtaagacttt ctttgtacta cctagtgatg acacactacg tagtgaagct ttcgagtact 1200

accatactct tgatgagagt tttcttggta ggtacatgtc tgctttaaac cacacaaaga 1260

aatggaaatt tcctcaagtt ggtggtttaa cttcaattaa atgggctgat aacaattgtt 1320

atttgtctag tgttttatta gcacttcaac agcttgaagt caaattcaat gcaccagcac 1380

ttcaagaggc ttattataga gcccgtgctg gtgatgctgc taacttttgt gcactcatac 1440

tc 1442

<210> SEQ ID NO 45
<211> LENGTH: 1050
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 45

atatgtctat gacatatgga cagcagtttg gtccaacata cttggatggt gctgatgtta 60

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caaaaattaa acctcatgta aatcatgagg gtaagacttt ctttgtacta cctagtgatg 120

acacactacg tagtgaagct ttcgagtact accatactct tgatgagagt tttcttggta 180

ggtacatgtc tgctttaaac cacacaaaga aatggaaatt tcctcaagtt ggtggtttaa 240

cttcaattaa atgggctgat aacaattgtt atttgtctag tgttttatta gcacttcaac 300

agcttgaagt caaattcaat gcaccagcac ttcaagaggc ttattataga gcccgtgctg 360

gtgatgctgc taacttttgt gcactcatac tcgcttacag taataaaact gttggcgagc 420

ttggtgatgt cagagaaact atgacccatc ttctacagca tgctaatttg gaatctgcaa 480

agcgagttct taatgtggtg tgtaaacatt gtggtcagaa aactactacc ttaacgggtg 540

tagaagctgt gatgtatatg ggtactctat cttatgataa tcttaagaca ggtgtttcca 600

ttccatgtgt gtgtggtcgt gatgctacac aatatctagt acaacaagag tcttcttttg 660

ttatgatgtc tgcaccacct gctgagtata aattacagca aggtacattc ttatgtgcga 720

atgagtacac tggtaactat cagtgtggtc attacactca tataactgct aaggagaccc 780

tctatcgtat tgacggagct caccttacaa agatgtcaga gtacaaagga ccagtgactg 840

atgttttcta caaggaaaca tcttacacta caaccatcaa gcctgtgtcg tataaactcg 900

atggagttac ttacacagag attgaaccaa aattggatgg gtattataaa aaggataatg 960

cttactatac agagcagcct atagaccttg taccaactca accattacca aatgcgagtt 1020

ttgataattt caaactcaca tgttctaaca 1050

<210> SEQ ID NO 46
<211> LENGTH: 1995
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 46

tttgtgcact catactcgct tacagtaata aaactgttgg cgagcttggt gatgtcagag 60

aaactatgac ccatcttcta cagcatgcta atttggaatc tgcaaagcga gttcttaatg 120

tggtgtgtaa acattgtggt cagaaaacta ctaccttaac gggtgtagaa gctgtgatgt 180

atatgggtac tctatcttat gataatctta agacaggtgt ttccattcca tgtgtgtgtg 240

gtcgtgatgc tacacaatat ctagtacaac aagagtcttc ttttgttatg atgtctgcac 300

cacctgctga gtataaatta cagcaaggta cattcttatg tgcgaatgag tacactggta 360

actatcagtg tggtcattac actcatataa ctgctaagga gaccctctat cgtattgacg 420

gagctcacct tacaaagatg tcagagtaca aaggaccagt gactgatgtt ttctacaagg 480

aaacatctta cactacaacc atcaagcctg tgtcgtataa actcgatgga gttacttaca 540

cagagattga accaaaattg gatgggtatt ataaaaagga taatgcttac tatacagagc 600

agcctataga ccttgtacca actcaaccat taccaaatgc gagttttgat aatttcaaac 660

tcacatgttc taacacaaaa tttgctgatg atttaaatca aatgacaggc ttcacaaagc 720

cagcttcacg agagctatct gtcacattct tcccagactt gaatggcgat gtagtggcta 780

ttgactatag acactattca gcgagtttca agaaaggtgc taaattactg cataagccaa 840

ttgtttggca cattaaccag gctacaacca agacaacgtt caaaccaaac acttggtgtt 900

tacgttgtct ttggagtaca aagccagtag atacttcaaa ttcatttgaa gttctggcag 960

tagaagacac acaaggaatg gacaatcttg cttgtgaaag tcaacaaccc acctctgaag 1020

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aagtagtgga aaatcctacc atacagaagg aagtcataga gtgtgacgtg aaaactaccg 1080

aagttgtagg caatgtcata cttaaaccat cagatgaagg tgttaaagta acacaagagt 1140

taggtcatga ggatcttatg gctgcttatg tggaaaacac aagcattacc attaagaaac 1200

ctaatgagct ttcactagcc ttaggtttaa aaacaattgc cactcatggt attgctgcaa 1260

ttaatagtgt tccttggagt aaaattttgg cttatgtcaa accattctta ggacaagcag 1320

caattacaac atcaaattgc gctaagagat tagcacaacg tgtgtttaac aattatatgc 1380

cttatgtgtt tacattattg ttccaattgt gtacttttac taaaagtacc aattctagaa 1440

ttagagcttc actacctaca actattgcta aaaatagtgt taagagtgtt gctaaattat 1500

gtttggatgc cggcattaat tatgtgaagt cacccaaatt ttctaaattg ttcacaatcg 1560

ctatgtggct attgttgtta agtatttgct taggttctct aatctgtgta actgctgctt 1620

ttggtgtact cttatctaat tttggtgctc cttcttattg taatggcgtt agagaattgt 1680

atcttaattc gtctaacgtt actactatgg atttctgtga aggttctttt ccttgcagca 1740

tttgtttaag tggattagac tcccttgatt cttatccagc tcttgaaacc attcaggtga 1800

cgatttcatc gtacaagcta gacttgacaa ttttaggtct ggccgctgag tgggttttgg 1860

catatatgtt gttcacaaaa ttcttttatt tattaggtct ttcagctata atgcaggtgt 1920

tctttggcta ttttgctagt catttcatca gcaattcttg gctcatgtgg tttatcatta 1980

gtattgtaca aatgg 1995

<210> SEQ ID NO 47
<211> LENGTH: 1884
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 47

aattcttggc tcatgtggtt tatcattagt attgtacaaa tggcacccgt ttctgcaatg 60

gttaggatgt acatcttctt tgcttctttc tactacatat ggaagagcta tgttcatatc 120

atggatggtt gcacctcttc gacttgcatg atgtgctata agcgcaatcg tgccacacgc 180

gttgagtgta caactattgt taatggcatg aagagatctt tctatgtcta tgcaaatgga 240

ggccgtggct tctgcaagac tcacaattgg aattgtctca attgtgacac attttgcact 300

ggtagtacat tcattagtga tgaagttgct cgtgatttgt cactccagtt taaaagacca 360

atcaacccta ctgaccagtc atcgtatatt gttgatagtg ttgctgtgaa aaatggcgcg 420

cttcacctct actttgacaa ggctggtcaa aagacctatg agagacatcc gctctcccat 480

tttgtcaatt tagacaattt gagagctaac aacactaaag gttcactgcc tattaatgtc 540

atagtttttg atggcaagtc caaatgcgac gagtctgctt ctaagtctgc ttctgtgtac 600

tacagtcagc tgatgtgcca acctattctg ttgcttgacc aagctcttgt atcagacgtt 660

ggagatagta ctgaagtttc cgttaagatg tttgatgctt atgtcgacac cttttcagca 720

acttttagtg ttcctatgga aaaacttaag gcacttgttg ctacagctca cagcgagtta 780

gcaaagggtg tagctttaga tggtgtcctt tctacattcg tgtcagctgc ccgacaaggt 840

gttgttgata ccgatgttga cacaaaggat gttattgaat gtctcaaact ttcacatcac 900

tctgacttag aagtgacagg tgacagttgt aacaatttca tgctcaccta taataaggtt 960

gaaaacatga cgcccagaga tcttggcgca tgtattgact gtaatgcaag gcatatcaat 1020

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gcccaagtag caaaaagtca caatgtttca ctcatctgga atgtaaaaga ctacatgtct 1080

ttatctgaac agctgcgtaa acaaattcgt agtgctgcca agaagaacaa catacctttt 1140

agactaactt gtgctacaac tagacaggtt gtcaatgtca taactactaa aatctcactc 1200

aagggtggta agattgttag tacttgtttt aaacttatgc ttaaggccac attattgtgc 1260

gttcttgctg cattggtttg ttatatcgtt atgccagtac atacattgtc aatccatgat 1320

ggttacacaa atgaaatcat tggttacaaa gccattcagg atggtgtcac tcgtgacatc 1380

atttctactg atgattgttt tgcaaataaa catgctggtt ttgacgcatg gtttagccag 1440

cgtggtggtt catacaaaaa tgacaaaagc tgccctgtag tagctgctat cattacaaga 1500

gagattggtt tcatagtgcc tggcttaccg ggtactgtgc tgagagcaat caatggtgac 1560

ttcttgcatt ttctacctcg tgtttttagt gctgttggca acatttgcta cacaccttcc 1620

aaactcattg agtatagtga ttttgctacc tctgcttgcg ttcttgctgc tgagtgtaca 1680

atttttaagg atgctatggg caaacctgtg ccatattgtt atgacactaa tttgctagag 1740

ggttctattt cttatagtga gcttcgtcca gacactcgtt atgtgcttat ggatggttcc 1800

atcatacagt ttcctaacac ttacctggag ggttctgtta gagtagtaac aacttttgat 1860

gctgagtact gtagacatgg taca 1884

<210> SEQ ID NO 48
<211> LENGTH: 2020
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 48

cactcgttat gtgcttatgg atggttccat catacagttt cctaacactt acctggaggg 60

ttctgttaga gtagtaacaa cttttgatgc tgagtactgt agacatggta catgcgaaag 120

gtcagaagta ggtatttgcc tatctaccag tggtagatgg gttcttaata atgagcatta 180

cagagctcta tcaggagttt tctgtggtgt tgatgcgatg aatctcatag ctaacatctt 240

tactcctctt gtgcaacctg tgggtgcttt agatgtgtct gcttcagtag tggctggtgg 300

tattattgcc atattggtga cttgtgctgc ctactacttt atgaaattca gacgtgtttt 360

tggtgagtac aaccatgttg ttgctgctaa tgcacttttg tttttgatgt ctttcactat 420

actctgtctg gtaccagctt acagctttct gccgggagtc tactcagtct tttacttgta 480

cttgacattc tatttcacca atgatgtttc attcttggct caccttcaat ggtttgccat 540

gttttctcct attgtgcctt tttggataac agcaatctat gtattctgta tttctctgaa 600

gcactgccat tggttcttta acaactatct taggaaaaga gtcatgttta atggagttac 660

atttagtacc ttcgaggagg ctgctttgtg taccttttg ctcaacaagg aaatgtacct 720

aaaattgcgt agcgagacac tgttgccact tacacagtat aacaggtatc ttgctctata 780

taacaagtac aagtatttca gtggagcctt agatactacc agctatcgtg aagcagcttg 840

ctgccactta gcaaaggctc taaatgactt tagcaactca ggtgctgatg ttctctacca 900

accaccacag acatcaatca cttctgctgt tctgcagagt ggttttagga aaatggcatt 960

cccgtcaggc aaagttgaag ggtgcatggt acaagtaacc tgtggaacta caactcttaa 1020

tggattgtgg ttggatgaca cagtatactg tccaagacat gtcatttgca cagcagaaga 1080

catgcttaat cctaactatg aagatctgct cattcgcaaa tccaaccata gctttcttgt 1140

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tcaggctggc aatgttcaac ttcgtgttat tggccattct atgcaaaatt gtctgcttag 1200

gcttaaagtt gatacttcta accctaagac acccaagtat aaatttgtcc gtatccaacc 1260

tggtcaaaca ttttcagttc tagcatgcta caatggttca ccatctggtg tttatcagtg 1320

tgccatgaga cctaatcata ccattaaagg ttctttcctt aatggatcat gtggtagtgt 1380

tggttttaac attgattatg attgcgtgtc tttctgctat atgcatcata tggagcttcc 1440

aacaggagta cacgctggta ctgacttaga aggtaaattc tatggtccat ttgttgacag 1500

acaaactgca caggctgcag gtacagacac aaccataaca ttaaatgttt tggcatggct 1560

gtatgctgct gttatcaatg gtgataggtg gtttcttaat agattcacca ctactttgaa 1620

tgactttaac cttgtggcaa tgaagtacaa ctatgaacct ttgacacaag atcatgttga 1680

catattggga cctctttctg ctcaaacagg aattgccgtc ttagatatgt gtgctgcttt 1740

gaaagagctg ctgcagaatg gtatgaatgg tcgtactatc cttggtagca ctattttaga 1800

agatgagttt acaccatttg atgttgttag acaatgctct ggtgttacct tccaaggtaa 1860

gttcaagaaa attgttaagg gcactcatca ttggatgctt ttaactttct tgacatcact 1920

attgattctt gttcaaagta cacagtggtc actgtttttc tttgtttacg agaatgcttt 1980

cttgccattt actcttggta ttatggcaat tgctgcatgt 2020

<210> SEQ ID NO 49
<211> LENGTH: 2040
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 49

agcatttcca gcctgaagac gtactgtagc agctaaactg cccagcacca tacctctatt 60

taggttgttt aagcctttga tgaagtacaa gtatttcact ttaggccctt ttggtgtgtc 120

tgtaacaaac ctacaaggtg gttccagttc tgtgtaaatt gtacctgtac catcactctt 180

agggaatcta gcccatttga gatcttggtg gtctgatagt aatgccagca caaacctacc 240

tcccttcgaa ttgttatagt aggcaagtgc attgtcatca gtacaagctg tttgtgtggt 300

accagccgca caggacatct gtcgtagtgc tactggactc agttcattat tctgtagttt 360

aacagctgag ttggctctta gagctgtaac aataagaggc caagccaaat ttggtgaatt 420

gtccatgtta atttcactaa gttgaacaat cttgctatcc gcatcaacaa cttgctggat 480

ttcccagagt gcagatgcat atgtaaaggt gttaccatca caagtgttct tgtaggtacc 540

ataatcaggg acaacaacca tgagtttggc tgctgtagtc aatggtatga tgttgagtgg 600

aacacaacca tcacgcgcat tgttgataat gttgttaagt gcatcattat caagcttcct 660

aagcatagtg aagagcattg tttgcatagc actagttact tttgccctct tgtcctcaga 720

tcttgcctgt ttgtacattt gggtcatagc ctgatctgcc atcttttcca acttgcgttg 780

catggcagca tcacggtcaa actcagattt agccacattc aaagatttct ttaacttttt 840

gagaacgact tcagaatcac cattagctac agcctgctca taggcctcct gggcagtggc 900

ataagcggca tatgatggta aagaactaaa ttctgaagca atagcctgaa gagtagcacg 960

gttatcgagc atttcctcgc acaacctatt aatgtctaca gcaccctgca tggatagcaa 1020

aacagacaaa agagaaacca tcttctcgaa agcttcagtt gtgtcttttg caagaagaat 1080

atcattgtgg agttgtacac attgtgccca caatttagaa gatgactcta ctctaagttg 1140

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ttgaagaacc gagagcagta ccacagatgt gcactttacg tcagacattt tagactgtac 1200

agtagcaacc ttgatacatg gtttacctcc aatacccaac aacttaatgt taagcttgaa 1260

agcatcaata ctactcttag gaggcaaaag cccctgggag ttcatatacc taaattcttg 1320

tgtagagacc aagtagtcat aaacaccaag agtaagcctg aagtaacggt tgagtaaaca 1380

gaaaaggcca aagtagcagc agcaacaata gcctaagaaa caataaacaa gcatgataca 1440

ctgtaaggtg ttgccagtaa taaataacaa tgggtaatac tcaacacaca caaacactat 1500

agctctagct aaaaacatga tagtcgtaac gacaccagaa tagttagagg ttacagaaat 1560

aactaaggcc cacatggaaa tagcttgatc taaagcatta ccatagtaga ctttgtaaac 1620

aagtgtaatg acattcatca gtgtccaaac acgtctagca gcatcatcat aaacagtgcg 1680

agctgtcatg agaataagca aaactaaagc tgaagcatac ataacacaat ccttaagcct 1740

ataaccagac aagctagtgt cagccaattc aagccatgtc atgatacgca tcacccagct 1800

agcaggcatg tagaccatat taaagtaagc aactgttgca agagaaggta acagaaacaa 1860

gcacaagaat gcgtgcttat gcttaacaag cagcatagca catgcagcaa ttgccataat 1920

accaagagta aatggcaaga aagcattctc gtaaacaaag aaaaacagtg accactgtgt 1980

actttgaaca agaatcaata gtgatgtcaa gaaagttaaa agcatccaat gatgagtgca 2040

<210> SEQ ID NO 50
<211> LENGTH: 2012
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 50

cttgtaggtt tgttacagac acaccaaaag ggcctaaagt gaaatacttg tacttcatca 60

aaggcttaaa caacctaaat agaggtatgg tgctgggcag tttagctgct acagtacgtc 120

ttcaggctgg aaatgctaca gaagtacctg ccaattcaac tgtgctttcc ttctgtgctt 180

ttgcagtaga ccctgctaaa gcatataagg attacctagc aagtggagga caaccaatca 240

ccaactgtgt gaagatgttg tgtacacaca ctggtacagg acaggcaatt actgtaacac 300

cagaagctaa catggaccaa gagtcctttg gtggtgcttc atgttgtctg tattgtagat 360

gccacattga ccatccaaat cctaaaggat tctgtgactt gaaaggtaag tacgtccaaa 420

tacctaccac ttgtgctaat gacccagtgg gttttacact tagaaacaca gtctgtaccg 480

tctgcggaat gtggaaaggt tatggctgta gttgtgacca actccgcgaa cccttgatgc 540

agtctgcgga tgcatcaacg tttttaaacg ggtttgcggt gtaagtgcag cccgtcttac 600

accgtgcggc acaggcacta gtactgatgt cgtctacagg gcttttgata tttacaacga 660

aaaagttgct ggttttgcaa agttcctaaa aactaattgc tgtcgcttcc aggagaagga 720

tgaggaaggc aatttattag actcttactt tgtagttaag aggcatacta tgtctaacta 780

ccaacatgaa gagactattt ataacttggt taaagattgt ccagcggttg ctgtccatga 840

ctttttcaag tttagagtag atggtgacat ggtaccacat atatcacgtc agcgtctaac 900

taaatacaca atggctgatt tagtctatgc tctacgtcat tttgatgagg gtaattgtga 960

tacattaaaa gaaatactcg tcacatacaa ttgctgtgat gatgattatt tcaataagaa 1020

ggattggtat gacttcgtag agaatcctga catcttacgc gtatatgcta acttaggtga 1080

gcgtgtacgc caatcattat taaagactgt acaattctgc gatgctatgc gtgatgcagg 1140

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tgatttcgta caagtagcac caggctgcgg agttcctatt gtggattcat attactcatt 1260

gctgatgccc atcctcactt tgactagggc attggctgct gagtcccata tggatgctga 1320

tctcgcaaaa ccacttatta agtgggattt gctgaaatat gattttacgg aagagagact 1380

ttgtctcttc gaccgttatt ttaaatattg ggaccagaca taccatccca attgtattaa 1440

ctgtttggat gataggtgta tccttcattg tgcaaacttt aatgtgttat tttctactgt 1500

gtttccacct acaagttttg gaccactagt aagaaaaata tttgtagatg gtgttccttt 1560

tgttgtttca actggatacc attttcgtga gttaggagtc gtacataatc aggatgtaaa 1620

cttacatagc tcgcgtctca gtttcaagga acttttagtg tatgctgctg atccagctat 1680

gcatgcagct tctggcaatt tattgctaga taaacgcact acatgctttt cagtagctgc 1740

actaacaaac aatgttgctt ttcaaactgt caaacccggt aattttaata aagactttta 1800

tgactttgct gtgtctaaag gtttctttaa ggaaggaagt tctgttgaac taaaacactt 1860

cttctttgct caggatggca acgctgctat cagtgattat gactattatc gttataatct 1920

gccaacaatg tgtgatatca gacaactcct attcgtagtt gaagttgttg ataaatactt 1980

tgattgttac gatggtggct gtattaatgc ca 2012

<210> SEQ ID NO 51
<211> LENGTH: 1877
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 51

gtacttcgcg tacagtggca ataccatatg acagcttaaa tgtttcctca gtggctttga 60

gcgtttctgc tgcgaaaagc ttgagtctct cagtacaagt gttggcaagt atgtaatcgc 120

cagcattagt ccaatcacat gttgctatcg cattgaagtc agtgacattg tcactgccta 180

cacatgtgtt tttgtataaa ccaaaaacct gaccattagc acataatgga aaactaatgg 240

gaggcttatg tgacttgcaa taatagctca tacctcctag atacagttgt gtcacatcag 300

tgacatcaca acctggggca ttgcaaacat agggattaac agacaacact aatttgtgtg 360

atgttgaaat gacatggtca tagcagcact tgcaacatag gaatggtctc ctaatacagg 420

caccgcaacg aagtgaagtc tgtgaattgc acaatacaca agcacctaca gcctgcaaga 480

ctgtatgtgg tgtgtacata gcctcataaa actcaggttc ccagtaccgt gaggtgttat 540

cattagttag cattacggaa tacatgtcca acatgtggcc agtaagctca tcatgtaact 600

ttctaatgta ttgtaaatac aagtgaaaga catcagcata ctcctgatta ggatgttttg 660

taagtgggta agcatcaata gccagtgaca cgaacctttc aatcataagt gtaccatctg 720

ttttgacaat atcatcgaca aaacagcctg cgcctaatat tcttgatgga tctgggtaag 780

gcaggtacac gtaatcatct ccttgtttaa ctagcattgt atgctgtgag caaaattcgt 840

gaggtccttt agtaaggtca gtctcagtcc aacattttgc ctcagacatg aacacattat 900

tttgataata aagaactgcc ttaaagttct taatgctagc tactaaacct tgagccgcat 960

agttactgtt atagcacaca acggcatcat cagaaagaat catcatggag aaatgtttac 1020

gcaggtaagc gtaaaactca tccacgaatt catgatcaac atccctattt ctatagagac 1080

actcatagag cctgtgttgt agattgcgga catacttgtc agctatctta ttaccatcag 1140

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ttgaaagaag tgcatttaca ttggctgtaa cagcttgaca aatgttaaag acactattag 1200

cataagcagt tgtagcatca ccggatgatg ttccacctgg tttaacatat agtgagccgc 1260

cacacatgac catctcactt aatacttgcg cacactcgtt agctaacctg tagaaacggt 1320

gtgataagtt acagcaagtg ttatgtttgc gagcaagaac aagagaggcc attatcctaa 1380

gcatgttagg catggctctg tcacattttg gataatccca acccataagg tgtggagttt 1440

ctacatcact gtaaacagtt tttaacatat tatgccagcc accgtaaaac ttgcttgttc 1500

caattaccac agtagctcct ctagtggcgg ctattgactt caataatttc tgatgaaact 1560

gtctatttgt catagtacta cagatagaga caccagctac ggtgcgagct ctattctttg 1620

cactaatggc atacttaaga ttcatttgag ttatagtagg gatgacatta cgcttagtat 1680

acgcgaaaag tgcatcttga tcctcataac tcattgagtc ataataaagt ctagccttac 1740

cccatttatt aaatgggaaa ccagctgatt tatccagatt gttaacgatt acttggttgg 1800

cattaataca gccaccatcg taacaatcaa agtatttatc aacaacttca actacgaata 1860

ggagttgtct gatatca 1877

<210> SEQ ID NO 52
<211> LENGTH: 2051
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 52

tcaggtccaa tcttgacaaa gtacttcatt gatgtaagct caaagccatg cgcccaaagg 60

acgaacacga ctctgtctga caatcctttc agtgtatcac tgagcatttg tactatctta 120

atacgcacta cattccaggg caagccttta tacatgagtg gtataagatg tttaaactgg 180

tcacctggtg gaggttttgc attaactctg gtgaattctg tgttattttc agtgtcaaca 240

taaccagtcg gtacagctac taagttaaca cctgtagaaa atcctagctg gagaggtagg 300

ttagtaccca cagcatctct agttgcatga cagccctcta catcaaagcc aatccacgca 360

cgaacgtgac gaatagcttc ttcgcgggtg ataaacatat tagggtaacc attgacttgg 420

taattcattt tgaaacccat catagagatg agtctacggt aggtcatgtc ctttggtatg 480

cctggtatgt caacacataa tccttcagtc ttgaacttta tatcaacgct gaggtgtgta 540

ggtgcctgtg taggatgaag accagtaatg atcttactac agtccttaaa aagtccagtt 600

acatttttctg cttgtaatgt agccacattg cgacgtggta tttctagact tgtaaattgc 660

agtttgtcat aaagatctct atcagacatt atgcacaaaa tgccaatttt tgcccttgtg 720

atagccacat tgaagcggtt gacattacaa gagtgtgctg tttcagtagt ttgtgtgaat 780

atgacatagt catattcaga accctgtgat gaatcaacag tctgcgtagg caatcctaag 840

atttttgaag ctacagcgtt ctgtgaatta taaggtgaga taaaaacagc ttttctccaa 900

gcaggattgc gtgtaagaaa ttctcttaca acgcctattt gaggtctgtt gattgcagat 960

gaaacatcat gtgtaataac acctttgtag aacattttga agcattgagc tgacttatcc 1020

ttgtgtgctt ttagcttatt gtcataaact aaagcactca cagtgtcaac aatttcagca 1080

ggacaacggc gacaagttcc aaggaacatg tctggaccta ttgttttcat aagtctgcac 1140

actgaattaa aatattctgg ttctagtgtg cctttagtca gcaatgtgcg gggggctggt 1200

aattgagcag gatcgccaat atagacgtag tgttttgcac gaagtctagc attgacaaca 1260

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ctcaagtcat aattagtagc catagagatt tcatcaaaga ctacaatgtc agcagttgtt 1320

tctggcaatg catttacagt gcagaaaaca tactgttcta gtgttgaatt cactttgaat 1380

ttatcaaaac actctacgcg cgcacgcgca ggtatgattc tactacattt atctatgggc 1440

aaatatttta atgccttttc acatagggca tcaacagctg catgagagca tgccgtatac 1500

actatgcgag cagatgggta atagagagca agtccgatgg caaaatgact cttaccagta 1560

ccaggtggtc cttggagtgt agagtacttt tgcatgccga ccttttgata atttgcaaca 1620

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tagtcacctt tttcaaaggt gtactctcca atctgtactt tactattttt agttacacgg 1860

taaccagtaa agacatagtt tctgttcaat ggtggtctag gttttccaac ctcccatgaa 1920

agatgcaatt ctctgtcaga gagtacttcg cgtacagtgg caataccata tgacagctta 1980

aatgtttcct cagtggcttt gagcgtttct gctgcgaaaa gcttgagtct ctcagtacaa 2040

gtgttggcaa g 2051

<210> SEQ ID NO 53
<211> LENGTH: 2075
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 53

tgcttgtagt tttgggtaga aggtttcaac atgtccatcc ttacaccaaa gcatgaatga 60

aatttcagca tagtcaattg taaccttgac cacttttgaa atcactgaca aatcttgtga 120

ctttattatc tcgacaaagt catcaagtaa aagatcaatc acagaacaca cacattttga 180

tgaacctgtt tgcgcatctg ttatgaagta atttttcact gtgctgtcca tagggataaa 240

atcctctaat ttaagtggtg aatcttgtga gcgcttggct aagcctatca ttaaatgaag 300

accgccaagt tgtccatgac tgaaatctcc ataaacgatg tgttcgaagg catagccctc 360

gagcttatat cgctgtatga attcatccat agcgagctcg agaaagtcag tttccatttg 420

tgatctgggc ttaaaatcct ctaagtctct gctctgagta aagtaggttt caggcaactg 480

ttgaataatg ccgtctactt tcttaaagta gttaaactgt gtttttactg attctccaat 540

taatgtgact ccattgacgc tagcttgtgc tggtcccttt gaaggtgtta gacctttgac 600

tgaaccttct gttattaaaa caccattacg ggcgtttcta aaaaggtcta cctgtccttc 660

cactctacca tcaaacaaga cagtaagtga agaacaagca ctctcagtag gtttcttggc 720

aatgtcagtc attgtgcaga cacctattgt agatacatgt gctggggctt ctcttttgta 780

gtcccagatt acagtattag cagcgatatc aacacccaaa ttattgagta tcttaatctc 840

tggcactggt ttaatgttac gcttagccca aagctcaaat gcaacattaa caggaagtgt 900

tgtcttattt tcaaagatct ccacatcaat accatctacc tttgtgtaaa cagcattatt 960

aatgatggaa acaggtgctt cgccggcgtg tccatcaaag tgtcctttat taacaacatt 1020

ataagccaca ttttctaaac tctgtaacct ggtaaatgta ttccacaggt tataagtatc 1080

aaattgtttg taaatccata ggctaaatcc agcagaaatc atcatattat atgcatccaa 1140

gtactgtcgg tactcatttg catggtgtct gcaaacagca ccacctaaat tgcatcgtgt 1200

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aatacacgta gcagatttga gtggaacata atcaatatcc gacactactt gtttgccatg 1260

agactcacaa ggactatcag aatagtaaaa gaaaggcaat tgctttaaat tagtaaatgc 1320

acttttatcg aaagctggag tgtggaatgc atgcttattc acatacaaac taccaccatc 1380

acagcctggt aagttcaagt ttgacaagac tcttgtgtca aacctacaca caattgcatt 1440

ggctgggtaa cgatcaacgt tacaattcca aaacaaacaa acaccatcag tgaatttatc 1500

gtgatgtgta gcataagaat agaagagttc ctctattttg taagctttgt cactacatgg 1560

ctgagcatcg tagaacttcc attctacttc agcctgaggc acacacttga tagcctttgg 1620

atttccaatg tcatgaagaa ctggaaactt atcagcaagc aatgcagact tcacaaccat 1680

gtgttgtact tttctgcaag cagaattaac cctcagttca tctcctataa tagggtattc 1740

aacagaccaa tcaacgcgct taacaaagca ctcatggact gctaaacatc tagtcatgat 1800

agcatcacaa ctagccacat gtgcatttcc atgtacctgg caatgttggt catggttact 1860

ctgaaggtta cccgtaaagc cccactgctg aacatcaatc ataaatgggt tatagacata 1920

gtcaaaaccc acagaatgat tccagcaggc ataagtatct gatgaagtag aaaagcaagt 1980

tgcacgtttg tcacacagac aacacgttct ttcaggtcca atcttgacaa agtacttcat 2040

tgatgtaagc tcaaagccat gcgcccaaag gacga 2075

<210> SEQ ID NO 54
<211> LENGTH: 1891
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 54

aagattcacc acttaaatta gaggatttta tccctatgga cagcacagtg aaaaattact 60

tcataacaga tgcgcaaaca ggttcatcaa aatgtgtgtg ttctgtgatt gatcttttac 120

ttgatgactt tgtcgagata ataaagtcac aagatttgtc agtgatttca aaagtggtca 180

aggttacaat tgactatgct gaaatttcat tcatgctttg gtgtaaggat ggacatgttg 240

aaaccttcta cccaaaacta caagcaagtc aagcgtggca accaggtgtt gcgatgccta 300

acttgtacaa gatgcaaaga atgcttcttg aaaagtgtga ccttcagaat tatggtgaaa 360

atgctgttat accaaaagga ataatgatga atgtcgcaaa gtatactcaa ctgtgtcaat 420

acttaaatac acttacttta gctgtaccct acaacatgag agttattcac tttggtgctg 480

gctctgataa aggagttgca ccaggtacag ctgtgctcag acaatggttg ccaactggca 540

cactacttgt cgattcagat cttaatgact tcgtctccga cgcagattct actttaattg 600

gagactgtgc aacagtacat acggctaata aatgggacct tattattagc gatatgtatg 660

accctaggac caaacatgtg acaaaagaga atgactctaa agaagggttt ttcacttatc 720

tgtgtggatt tataaagcaa aaactagccc tgggtggttc tatagctgta aagataacag 780

agcattcttg gaatgctgac ctttacaagc ttatgggcca tttctcatgg tggacagctt 840

ttgttacaaa tgtaaatgca tcatcatcgg aagcattttt aattggggct aactatcttg 900

gcaagccgaa ggaacaaatt gatggctata ccatgcatgc taactacatt ttctggagga 960

acacaaatcc tatccagttg tcttcctatt cactctttga catgagcaaa tttcctctta 1020

aattaagagg aactgctgta atgtctctta aggagaatca aatcaatgat atgatttatt 1080

ctcttctgga aaaaggtagg cttatcatta gagaaaacaa cagagttgtg gtttcaagtg 1140

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atattcttgt taacaactaa acgaacatgt ttattttctt attatttctt actctcacta 1200

gtggtagtga ccttgaccgg tgcaccactt ttgatgatgt tcaagctcct aattacactc 1260

aacatacttc atctatgagg ggggtttact atcctgatga aatttttaga tcagacactc 1320

tttatttaac tcaggattta tttcttccat tttattctaa tgttacaggg tttcatacta 1380

ttaatcatac gtttggcaac cctgtcatac cttttaagga tggtatttat tttgctgcca 1440

cagagaaatc aaatgttgtc cgtggttggg tttttggttc taccatgaac aacaagtcac 1500

agtcggtgat tattattaac aattctacta atgttgttat acgagcatgt aactttgaat 1560

tgtgtgacaa ccctttcttt gctgtttcta aacccatggg tacacagaca catactatga 1620

tattcgataa tgcatttaat tgcactttcg agtacatatc tgatgccttt tcgcttgatg 1680

tttcagaaaa gtcaggtaat tttaaacact tacgagagtt tgtgtttaaa aataaagatg 1740

ggtttctcta tgtttataag ggctatcaac ctatagatgt agttcgtgat ctaccttctg 1800

gttttaacac tttgaaacct atttttaagt tgcctcttgg tattaacatt acaaatttta 1860

gagccattct tacagccttt tcacctgctc a 1891

<210> SEQ ID NO 55
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: N sens primer

<400> SEQUENCE: 55

cccatatgtc tgataatgga ccccaatcaa ac 32

<210> SEQ ID NO 56
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: N antisens primer

<400> SEQUENCE: 56

cccccgggtg cctgagttga atcagcagaa gc 32

<210> SEQ ID NO 57
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sc sens primer

<400> SEQUENCE: 57

cccatatgag tgaccttgac cggtgcacca c 31

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SL sens primer

<400> SEQUENCE: 58

cccatatgaa accttgcacc ccacctgctc 30

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<210> SEQ ID NO 59
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Sc and SL antisens primer

<400> SEQUENCE: 59

ccccgggtt taatatattg ctcatatttt ccc 33

<210> SEQ ID NO 60
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Sens set 1 primer

<400> SEQUENCE: 60

ggcatcgtat gggttg 16

<210> SEQ ID NO 61
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Antisens set 2 (28774-28759) primer

<400> SEQUENCE: 61

cagtttcacc acctcc 16

<210> SEQ ID NO 62
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Sens set 2 (28375-28390) primer

<400> SEQUENCE: 62

ggctactacc gaagag 16

<210> SEQ ID NO 63
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Antisens set 2 (28702-28687)primer

<400> SEQUENCE: 63

aattaccgcg actacg 16

<210> SEQ ID NO 64
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Probe 1/set 1 (28561-28586)

<400> SEQUENCE: 64

ggcacccgca atcctaataa caatgc 26

<210> SEQ ID NO 65
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Probe 2/set 1 (28588-28608)

<400> SEQUENCE: 65

gccaccgtgc tacaacttcc t 21

<210> SEQ ID NO 66
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Probe 1/set 2 /probe N/FL (28541-28563)

<400> SEQUENCE: 66

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atacacccaa agaccacatt ggc 23

<210> SEQ ID NO 67
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Probe 2/set 2/probe SARS/N/LC705 (28565-28589)

<400> SEQUENCE: 67

cccgcaatcc taataacaat gctgc 25

<210> SEQ ID NO 68
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Anchor primer 14T

<400> SEQUENCE: 68

agatgaattc ggtacctttt ttttttttt 30

<210> SEQ ID NO 69
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: M2-14 peptide

<400> SEQUENCE: 69

Ala Asp Asn Gly Thr Ile Thr Val Glu Glu Leu Lys Gln
1 5 10

<210> SEQ ID NO 70
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: E1-12 peptide

<400> SEQUENCE: 70

Met Tyr Ser Phe Val Ser Glu Glu Thr Gly Thr Leu
1 5 10

<210> SEQ ID NO 71
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: E53-72 peptide

<400> SEQUENCE: 71

Lys Pro Thr Val Tyr Val Tyr Ser Arg Val Lys Asn Leu Asn Ser Ser
1 5 10 15

Glu Gly Val Pro Asp Leu Leu Val
20

<210> SEQ ID NO 72
<211> LENGTH: 153
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 72

gatattaggt ttttacctac ccaggaaaag ccaaccaacc tcgatctctt gtagatctgt 60

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tctctaaacg aactttaaaa tctgtgtagc tgtcgctcgg ctgcatgcct agtgcaccta 120

cgcagtataa acaataataa attttactgt cgt 153

<210> SEQ ID NO 73
<211> LENGTH: 410
<212> TYPE: DNA
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 73

ttctccagac aacttcaaaa ttccatgagt ggagcttctg ctgattcaac tcaggcataa 60

acactcatga tgaccacaca aggcagatgg gctatgtaaa cgttttcgca attccgttta 120

cgatacatag tctactcttg tgcagaatga attctcgtaa ctaaacagca caagtaggtt 180

tagttaactt taatctcaca tagcaatctt taatcaatgt gtaacattag ggaggacttg 240

aaagagccac cacattttca tcgaggccac gcggagtacg atcgagggta cagtgaataa 300

tgctagggag agctgcctat atggaagagc cctaatgtgt aaaattaatt ttagtagtgc 360

tatccccatg tgattttaat agcttcttag gagaatgaca aaaaaaaaaa 410

<210> SEQ ID NO 74
<211> LENGTH: 4382
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 74

Met Glu Ser Leu Val Leu Gly Val Asn Glu Lys Thr His Val Gln Leu
1 5 10 15

Ser Leu Pro Val Leu Gln Val Arg Asp Val Leu Val Arg Gly Phe Gly
20 25 30

Asp Ser Val Glu Glu Ala Leu Ser Glu Ala Arg Glu His Leu Lys Asn
35 40 45

Gly Thr Cys Gly Leu Val Glu Leu Glu Lys Gly Val Leu Pro Gln Leu
50 55 60

Glu Gln Pro Tyr Val Phe Ile Lys Arg Ser Asp Ala Leu Ser Thr Asn
65 70 75 80

His Gly His Lys Val Val Glu Leu Val Ala Glu Met Asp Gly Ile Gln
85 90 95

Tyr Gly Arg Ser Gly Ile Thr Leu Gly Val Leu Val Pro His Val Gly
100 105 110

Glu Thr Pro Ile Ala Tyr Arg Asn Val Leu Leu Arg Lys Asn Gly Asn
115 120 125

Lys Gly Ala Gly Gly His Ser Tyr Gly Ile Asp Leu Lys Ser Tyr Asp
130 135 140

Leu Gly Asp Glu Leu Gly Thr Asp Pro Ile Glu Asp Tyr Glu Gln Asn
145 150 155 160

Trp Asn Thr Lys His Gly Ser Gly Ala Leu Arg Glu Leu Thr Arg Glu
165 170 175

Leu Asn Gly Gly Ala Val Thr Arg Tyr Val Asp Asn Asn Phe Cys Gly
180 185 190

Pro Asp Gly Tyr Pro Leu Asp Cys Ile Lys Asp Phe Leu Ala Arg Ala
195 200 205

Gly Lys Ser Met Cys Thr Leu Ser Glu Gln Leu Asp Tyr Ile Glu Ser
210 215 220

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Lys Arg Gly Val Tyr Cys Cys Arg Asp His Glu His Glu Ile Ala Trp
225                 230                 235                 240

Phe Thr Glu Arg Ser Asp Lys Ser Tyr Glu His Gln Thr Pro Phe Glu
                245                 250                 255

Ile Lys Ser Ala Lys Lys Phe Asp Thr Phe Lys Gly Glu Cys Pro Lys
            260                 265                 270

Phe Val Phe Pro Leu Asn Ser Lys Val Lys Val Ile Gln Pro Arg Val
        275                 280                 285

Glu Lys Lys Lys Thr Glu Gly Phe Met Gly Arg Ile Arg Ser Val Tyr
    290                 295                 300

Pro Val Ala Ser Pro Gln Glu Cys Asn Asn Met His Leu Ser Thr Leu
305                 310                 315                 320

Met Lys Cys Asn His Cys Asp Glu Val Ser Trp Gln Thr Cys Asp Phe
                325                 330                 335

Leu Lys Ala Thr Cys Glu His Cys Gly Thr Glu Asn Leu Val Ile Glu
            340                 345                 350

Gly Pro Thr Thr Cys Gly Tyr Leu Pro Thr Asn Ala Val Val Lys Met
        355                 360                 365

Pro Cys Pro Ala Cys Gln Asp Pro Glu Ile Gly Pro Glu His Ser Val
    370                 375                 380

Ala Asp Tyr His Asn His Ser Asn Ile Glu Thr Arg Leu Arg Lys Gly
385                 390                 395                 400

Gly Arg Thr Arg Cys Phe Gly Gly Cys Val Phe Ala Tyr Val Gly Cys
                405                 410                 415

Tyr Asn Lys Arg Ala Tyr Trp Val Pro Arg Ala Ser Ala Asp Ile Gly
            420                 425                 430

Ser Gly His Thr Gly Ile Thr Gly Asp Asn Val Glu Thr Leu Asn Glu
        435                 440                 445

Asp Leu Leu Glu Ile Leu Ser Arg Glu Arg Val Asn Ile Asn Ile Val
    450                 455                 460

Gly Asp Phe His Leu Asn Glu Glu Val Ala Ile Ile Leu Ala Ser Phe
465                 470                 475                 480

Ser Ala Ser Thr Ser Ala Phe Ile Asp Thr Ile Lys Ser Leu Asp Tyr
                485                 490                 495

Lys Ser Phe Lys Thr Ile Val Glu Ser Cys Gly Asn Tyr Lys Val Thr
            500                 505                 510

Lys Gly Lys Pro Val Lys Gly Ala Trp Asn Ile Gly Gln Gln Arg Ser
        515                 520                 525

Val Leu Thr Pro Leu Cys Gly Phe Pro Ser Gln Ala Ala Gly Val Ile
    530                 535                 540

Arg Ser Ile Phe Ala Arg Thr Leu Asp Ala Ala Asn His Ser Ile Pro
545                 550                 555                 560

Asp Leu Gln Arg Ala Ala Val Thr Ile Leu Asp Gly Ile Ser Glu Gln
                565                 570                 575

Ser Leu Arg Leu Val Asp Ala Met Val Tyr Thr Ser Asp Leu Leu Thr
            580                 585                 590

Asn Ser Val Ile Ile Met Ala Tyr Val Thr Gly Gly Leu Val Gln Gln
        595                 600                 605

Thr Ser Gln Trp Leu Ser Asn Leu Leu Gly Thr Thr Val Glu Lys Leu
    610                 615                 620
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Arg Pro Ile Phe Glu Trp Ile Glu Ala Lys Leu Ser Ala Gly Val Glu
625                 630                 635                 640

Phe Leu Lys Asp Ala Trp Glu Ile Leu Lys Phe Leu Ile Thr Gly Val
                645                 650                 655

Phe Asp Ile Val Lys Gly Gln Ile Gln Val Ala Ser Asp Asn Ile Lys
            660                 665                 670

Asp Cys Val Lys Cys Phe Ile Asp Val Val Asn Lys Ala Leu Glu Met
        675                 680                 685

Cys Ile Asp Gln Val Thr Ile Ala Gly Ala Lys Leu Arg Ser Leu Asn
    690                 695                 700

Leu Gly Glu Val Phe Ile Ala Gln Ser Lys Gly Leu Tyr Arg Gln Cys
705                 710                 715                 720

Ile Arg Gly Lys Glu Gln Leu Gln Leu Leu Met Pro Leu Lys Ala Pro
                725                 730                 735

Lys Glu Val Thr Phe Leu Glu Gly Asp Ser His Asp Thr Val Leu Thr
            740                 745                 750

Ser Glu Glu Val Val Leu Lys Asn Gly Glu Leu Glu Ala Leu Glu Thr
        755                 760                 765

Pro Val Asp Ser Phe Thr Asn Gly Ala Ile Val Gly Thr Pro Val Cys
    770                 775                 780

Val Asn Gly Leu Met Leu Leu Glu Ile Lys Asp Lys Glu Gln Tyr Cys
785                 790                 795                 800

Ala Leu Ser Pro Gly Leu Leu Ala Thr Asn Asn Val Phe Arg Leu Lys
                805                 810                 815

Gly Gly Ala Pro Ile Lys Gly Val Thr Phe Gly Glu Asp Thr Val Trp
            820                 825                 830

Glu Val Gln Gly Tyr Lys Asn Val Arg Ile Thr Phe Glu Leu Asp Glu
        835                 840                 845

Arg Val Asp Lys Val Leu Asn Glu Lys Cys Ser Val Tyr Thr Val Glu
    850                 855                 860

Ser Gly Thr Glu Val Thr Glu Phe Ala Cys Val Val Ala Glu Ala Val
865                 870                 875                 880

Val Lys Thr Leu Gln Pro Val Ser Asp Leu Leu Thr Asn Met Gly Ile
                885                 890                 895

Asp Leu Asp Glu Trp Ser Val Ala Thr Phe Tyr Leu Phe Asp Asp Ala
            900                 905                 910

Gly Glu Glu Asn Phe Ser Ser Arg Met Tyr Cys Ser Phe Tyr Pro Pro
        915                 920                 925

Asp Glu Glu Glu Glu Asp Asp Ala Glu Cys Glu Glu Glu Glu Ile Asp
    930                 935                 940

Glu Thr Cys Glu His Glu Tyr Gly Thr Glu Asp Asp Tyr Gln Gly Leu
945                 950                 955                 960

Pro Leu Glu Phe Gly Ala Ser Ala Glu Thr Val Arg Val Glu Glu Glu
                965                 970                 975

Glu Glu Glu Asp Trp Leu Asp Asp Thr Thr Glu Gln Ser Glu Ile Glu
            980                 985                 990

Pro Glu Pro Glu Pro Thr Pro Glu Glu Pro Val Asn Gln Phe Thr Gly
        995                 1000                1005

Tyr Leu  Lys Leu Thr Asp Asn  Val Ala Ile Lys Cys  Val Asp Ile
    1010                1015                1020

Val Lys  Glu Ala Gln Ser Ala  Asn Pro Met Val Ile  Val Asn Ala
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    1025                1030                1035

Ala Asn  Ile His Leu Lys His  Gly Gly Gly Val Ala  Gly Ala Leu
    1040                1045                1050

Asn Lys  Ala Thr Asn Gly Ala  Met Gln Lys Glu Ser  Asp Asp Tyr
    1055                1060                1065

Ile Lys  Leu Asn Gly Pro Leu  Thr Val Gly Gly Ser  Cys Leu Leu
    1070                1075                1080

Ser Gly  His Asn Leu Ala Lys  Lys Cys Leu His Val  Val Gly Pro
    1085                1090                1095

Asn Leu  Asn Ala Gly Glu Asp  Ile Gln Leu Leu Lys  Ala Ala Tyr
    1100                1105                1110

Glu Asn  Phe Asn Ser Gln Asp  Ile Leu Leu Ala Pro  Leu Leu Ser
    1115                1120                1125

Ala Gly  Ile Phe Gly Ala Lys  Pro Leu Gln Ser Leu  Gln Val Cys
    1130                1135                1140

Val Gln  Thr Val Arg Thr Gln  Val Tyr Ile Ala Val  Asn Asp Lys
    1145                1150                1155

Ala Leu  Tyr Glu Gln Val Val  Met Asp Tyr Leu Asp  Asn Leu Lys
    1160                1165                1170

Pro Arg  Val Glu Ala Pro Lys  Gln Glu Glu Pro Pro  Asn Thr Glu
    1175                1180                1185

Asp Ser  Lys Thr Glu Glu Lys  Ser Val Val Gln Lys  Pro Val Asp
    1190                1195                1200

Val Lys  Pro Lys Ile Lys Ala  Cys Ile Asp Glu Val  Thr Thr Thr
    1205                1210                1215

Leu Glu  Glu Thr Lys Phe Leu  Thr Asn Lys Leu Leu  Leu Phe Ala
    1220                1225                1230

Asp Ile  Asn Gly Lys Leu Tyr  His Asp Ser Gln Asn  Met Leu Arg
    1235                1240                1245

Gly Glu  Asp Met Ser Phe Leu  Glu Lys Asp Ala Pro  Tyr Met Val
    1250                1255                1260

Gly Asp  Val Ile Thr Ser Gly  Asp Ile Thr Cys Val  Val Ile Pro
    1265                1270                1275

Ser Lys  Lys Ala Gly Gly Thr  Thr Glu Met Leu Ser  Arg Ala Leu
    1280                1285                1290

Lys Lys  Val Pro Val Asp Glu  Tyr Ile Thr Thr Tyr  Pro Gly Gln
    1295                1300                1305

Gly Cys  Ala Gly Tyr Thr Leu  Glu Glu Ala Lys Thr  Ala Leu Lys
    1310                1315                1320

Lys Cys  Lys Ser Ala Phe Tyr  Val Leu Pro Ser Glu  Ala Pro Asn
    1325                1330                1335

Ala Lys  Glu Glu Ile Leu Gly  Thr Val Ser Trp Asn  Leu Arg Glu
    1340                1345                1350

Met Leu  Ala His Ala Glu Glu  Thr Arg Lys Leu Met  Pro Ile Cys
    1355                1360                1365

Met Asp  Val Arg Ala Ile Met  Ala Thr Ile Gln Arg  Lys Tyr Lys
    1370                1375                1380

Gly Ile  Lys Ile Gln Glu Gly  Ile Val Asp Tyr Gly  Val Arg Phe
    1385                1390                1395

Phe Phe  Tyr Thr Ser Lys Glu  Pro Val Ala Ser Ile  Ile Thr Lys
    1400                1405                1410
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Leu Asn  Ser Leu Asn Glu Pro  Leu Val Thr Met Pro  Ile Gly Tyr
    1415                 1420                 1425

Val Thr  His Gly Phe Asn Leu  Glu Glu Ala Ala Arg  Cys Met Arg
    1430                 1435                 1440

Ser Leu  Lys Ala Pro Ala Val  Val Ser Val Ser Ser  Pro Asp Ala
    1445                 1450                 1455

Val Thr  Thr Tyr Asn Gly Tyr  Leu Thr Ser Ser Ser  Lys Thr Ser
    1460                 1465                 1470

Glu Glu  His Phe Val Glu Thr  Val Ser Leu Ala Gly  Ser Tyr Arg
    1475                 1480                 1485

Asp Trp  Ser Tyr Ser Gly Gln  Arg Thr Glu Leu Gly  Val Glu Phe
    1490                 1495                 1500

Leu Lys  Arg Gly Asp Lys Ile  Val Tyr His Thr Leu  Glu Ser Pro
    1505                 1510                 1515

Val Glu  Phe His Leu Asp Gly  Glu Val Leu Ser Leu  Asp Lys Leu
    1520                 1525                 1530

Lys Ser  Leu Leu Ser Leu Arg  Glu Val Lys Thr Ile  Lys Val Phe
    1535                 1540                 1545

Thr Thr  Val Asp Asn Thr Asn  Leu His Thr Gln Leu  Val Asp Met
    1550                 1555                 1560

Ser Met  Thr Tyr Gly Gln Gln  Phe Gly Pro Thr Tyr  Leu Asp Gly
    1565                 1570                 1575

Ala Asp  Val Thr Lys Ile Lys  Pro His Val Asn His  Glu Gly Lys
    1580                 1585                 1590

Thr Phe  Phe Val Leu Pro Ser  Asp Asp Thr Leu Arg  Ser Glu Ala
    1595                 1600                 1605

Phe Glu  Tyr Tyr His Thr Leu  Asp Glu Ser Phe Leu  Gly Arg Tyr
    1610                 1615                 1620

Met Ser  Ala Leu Asn His Thr  Lys Lys Trp Lys Phe  Pro Gln Val
    1625                 1630                 1635

Gly Gly  Leu Thr Ser Ile Lys  Trp Ala Asp Asn Asn  Cys Tyr Leu
    1640                 1645                 1650

Ser Ser  Val Leu Leu Ala Leu  Gln Gln Leu Glu Val  Lys Phe Asn
    1655                 1660                 1665

Ala Pro  Ala Leu Gln Glu Ala  Tyr Tyr Arg Ala Arg  Ala Gly Asp
    1670                 1675                 1680

Ala Ala  Asn Phe Cys Ala Leu  Ile Leu Ala Tyr Ser  Asn Lys Thr
    1685                 1690                 1695

Val Gly  Glu Leu Gly Asp Val  Arg Glu Thr Met Thr  His Leu Leu
    1700                 1705                 1710

Gln His  Ala Asn Leu Glu Ser  Ala Lys Arg Val Leu  Asn Val Val
    1715                 1720                 1725

Cys Lys  His Cys Gly Gln Lys  Thr Thr Thr Leu Thr  Gly Val Glu
    1730                 1735                 1740

Ala Val  Met Tyr Met Gly Thr  Leu Ser Tyr Asp Asn  Leu Lys Thr
    1745                 1750                 1755

Gly Val  Ser Ile Pro Cys Val  Cys Gly Arg Asp Ala  Thr Gln Tyr
    1760                 1765                 1770

Leu Val  Gln Gln Glu Ser Ser  Phe Val Met Met Ser  Ala Pro Pro
    1775                 1780                 1785
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Ala Glu  Tyr Lys Leu Gln Gln  Gly Thr Phe Leu Cys  Ala Asn Glu
    1790                 1795                 1800

Tyr Thr  Gly Asn Tyr Gln Cys  Gly His Tyr Thr His  Ile Thr Ala
    1805                 1810                 1815

Lys Glu  Thr Leu Tyr Arg Ile  Asp Gly Ala His Leu  Thr Lys Met
    1820                 1825                 1830

Ser Glu  Tyr Lys Gly Pro Val  Thr Asp Val Phe Tyr  Lys Glu Thr
    1835                 1840                 1845

Ser Tyr  Thr Thr Thr Ile Lys  Pro Val Ser Tyr Lys  Leu Asp Gly
    1850                 1855                 1860

Val Thr  Tyr Thr Glu Ile Glu  Pro Lys Leu Asp Gly  Tyr Tyr Lys
    1865                 1870                 1875

Lys Asp  Asn Ala Tyr Tyr Thr  Glu Gln Pro Ile Asp  Leu Val Pro
    1880                 1885                 1890

Thr Gln  Pro Leu Pro Asn Ala  Ser Phe Asp Asn Phe  Lys Leu Thr
    1895                 1900                 1905

Cys Ser  Asn Thr Lys Phe Ala  Asp Asp Leu Asn Gln  Met Thr Gly
    1910                 1915                 1920

Phe Thr  Lys Pro Ala Ser Arg  Glu Leu Ser Val Thr  Phe Phe Pro
    1925                 1930                 1935

Asp Leu  Asn Gly Asp Val Val  Ala Ile Asp Tyr Arg  His Tyr Ser
    1940                 1945                 1950

Ala Ser  Phe Lys Lys Gly Ala  Lys Leu Leu His Lys  Pro Ile Val
    1955                 1960                 1965

Trp His  Ile Asn Gln Ala Thr  Thr Lys Thr Thr Phe  Lys Pro Asn
    1970                 1975                 1980

Thr Trp  Cys Leu Arg Cys Leu  Trp Ser Thr Lys Pro  Val Asp Thr
    1985                 1990                 1995

Ser Asn  Ser Phe Glu Val Leu  Ala Val Glu Asp Thr  Gln Gly Met
    2000                 2005                 2010

Asp Asn  Leu Ala Cys Glu Ser  Gln Gln Pro Thr Ser  Glu Glu Val
    2015                 2020                 2025

Val Glu  Asn Pro Thr Ile Gln  Lys Glu Val Ile Glu  Cys Asp Val
    2030                 2035                 2040

Lys Thr  Thr Glu Val Val Gly  Asn Val Ile Leu Lys  Pro Ser Asp
    2045                 2050                 2055

Glu Gly  Val Lys Val Thr Gln  Glu Leu Gly His Glu  Asp Leu Met
    2060                 2065                 2070

Ala Ala  Tyr Val Glu Asn Thr  Ser Ile Thr Ile Lys  Lys Pro Asn
    2075                 2080                 2085

Glu Leu  Ser Leu Ala Leu Gly  Leu Lys Thr Ile Ala  Thr His Gly
    2090                 2095                 2100

Ile Ala  Ala Ile Asn Ser Val  Pro Trp Ser Lys Ile  Leu Ala Tyr
    2105                 2110                 2115

Val Lys  Pro Phe Leu Gly Gln  Ala Ala Ile Thr Thr  Ser Asn Cys
    2120                 2125                 2130

Ala Lys  Arg Leu Ala Gln Arg  Val Phe Asn Asn Tyr  Met Pro Tyr
    2135                 2140                 2145

Val Phe  Thr Leu Leu Phe Gln  Leu Cys Thr Phe Thr  Lys Ser Thr
    2150                 2155                 2160

Asn Ser  Arg Ile Arg Ala Ser  Leu Pro Thr Thr Ile  Ala Lys Asn
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    2165                2170                2175

Ser Val  Lys Ser Val Ala Lys  Leu Cys Leu Asp Ala  Gly Ile Asn
    2180                2185                2190

Tyr Val  Lys Ser Pro Lys Phe  Ser Lys Leu Phe Thr  Ile Ala Met
    2195                2200                2205

Trp Leu  Leu Leu Leu Ser Ile  Cys Leu Gly Ser Leu  Ile Cys Val
    2210                2215                2220

Thr Ala  Ala Phe Gly Val Leu  Leu Ser Asn Phe Gly  Ala Pro Ser
    2225                2230                2235

Tyr Cys  Asn Gly Val Arg Glu  Leu Tyr Leu Asn Ser  Ser Asn Val
    2240                2245                2250

Thr Thr  Met Asp Phe Cys Glu  Gly Ser Phe Pro Cys  Ser Ile Cys
    2255                2260                2265

Leu Ser  Gly Leu Asp Ser Leu  Asp Ser Tyr Pro Ala  Leu Glu Thr
    2270                2275                2280

Ile Gln  Val Thr Ile Ser Ser  Tyr Lys Leu Asp Leu  Thr Ile Leu
    2285                2290                2295

Gly Leu  Ala Ala Glu Trp Val  Leu Ala Tyr Met Leu  Phe Thr Lys
    2300                2305                2310

Phe Phe  Tyr Leu Leu Gly Leu  Ser Ala Ile Met Gln  Val Phe Phe
    2315                2320                2325

Gly Tyr  Phe Ala Ser His Phe  Ile Ser Asn Ser Trp  Leu Met Trp
    2330                2335                2340

Phe Ile  Ile Ser Ile Val Gln  Met Ala Pro Val Ser  Ala Met Val
    2345                2350                2355

Arg Met  Tyr Ile Phe Phe Ala  Ser Phe Tyr Tyr Ile  Trp Lys Ser
    2360                2365                2370

Tyr Val  His Ile Met Asp Gly  Cys Thr Ser Ser Thr  Cys Met Met
    2375                2380                2385

Cys Tyr  Lys Arg Asn Arg Ala  Thr Arg Val Glu Cys  Thr Thr Ile
    2390                2395                2400

Val Asn  Gly Met Lys Arg Ser  Phe Tyr Val Tyr Ala  Asn Gly Gly
    2405                2410                2415

Arg Gly  Phe Cys Lys Thr His  Asn Trp Asn Cys Leu  Asn Cys Asp
    2420                2425                2430

Thr Phe  Cys Thr Gly Ser Thr  Phe Ile Ser Asp Glu  Val Ala Arg
    2435                2440                2445

Asp Leu  Ser Leu Gln Phe Lys  Arg Pro Ile Asn Pro  Thr Asp Gln
    2450                2455                2460

Ser Ser  Tyr Ile Val Asp Ser  Val Ala Val Lys Asn  Gly Ala Leu
    2465                2470                2475

His Leu  Tyr Phe Asp Lys Ala  Gly Gln Lys Thr Tyr  Glu Arg His
    2480                2485                2490

Pro Leu  Ser His Phe Val Asn  Leu Asp Asn Leu Arg  Ala Asn Asn
    2495                2500                2505

Thr Lys  Gly Ser Leu Pro Ile  Asn Val Ile Val Phe  Asp Gly Lys
    2510                2515                2520

Ser Lys  Cys Asp Glu Ser Ala  Ser Lys Ser Ala Ser  Val Tyr Tyr
    2525                2530                2535

Ser Gln  Leu Met Cys Gln Pro  Ile Leu Leu Leu Asp  Gln Ala Leu
    2540                2545                2550
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Val Ser  Asp Val Gly Asp Ser  Thr Glu Val Ser Val  Lys Met Phe
    2555                 2560                 2565

Asp Ala  Tyr Val Asp Thr Phe  Ser Ala Thr Phe Ser  Val Pro Met
    2570                 2575                 2580

Glu Lys  Leu Lys Ala Leu Val  Ala Thr Ala His Ser  Glu Leu Ala
    2585                 2590                 2595

Lys Gly  Val Ala Leu Asp Gly  Val Leu Ser Thr Phe  Val Ser Ala
    2600                 2605                 2610

Ala Arg  Gln Gly Val Val Asp  Thr Asp Val Asp Thr  Lys Asp Val
    2615                 2620                 2625

Ile Glu  Cys Leu Lys Leu Ser  His His Ser Asp Leu  Glu Val Thr
    2630                 2635                 2640

Gly Asp  Ser Cys Asn Asn Phe  Met Leu Thr Tyr Asn  Lys Val Glu
    2645                 2650                 2655

Asn Met  Thr Pro Arg Asp Leu  Gly Ala Cys Ile Asp  Cys Asn Ala
    2660                 2665                 2670

Arg His  Ile Asn Ala Gln Val  Ala Lys Ser His Asn  Val Ser Leu
    2675                 2680                 2685

Ile Trp  Asn Val Lys Asp Tyr  Met Ser Leu Ser Glu  Gln Leu Arg
    2690                 2695                 2700

Lys Gln  Ile Arg Ser Ala Ala  Lys Lys Asn Asn Ile  Pro Phe Arg
    2705                 2710                 2715

Leu Thr  Cys Ala Thr Thr Arg  Gln Val Val Asn Val  Ile Thr Thr
    2720                 2725                 2730

Lys Ile  Ser Leu Lys Gly Gly  Lys Ile Val Ser Thr  Cys Phe Lys
    2735                 2740                 2745

Leu Met  Leu Lys Ala Thr Leu  Leu Cys Val Leu Ala  Ala Leu Val
    2750                 2755                 2760

Cys Tyr  Ile Val Met Pro Val  His Thr Leu Ser Ile  His Asp Gly
    2765                 2770                 2775

Tyr Thr  Asn Glu Ile Ile Gly  Tyr Lys Ala Ile Gln  Asp Gly Val
    2780                 2785                 2790

Thr Arg  Asp Ile Ile Ser Thr  Asp Asp Cys Phe Ala  Asn Lys His
    2795                 2800                 2805

Ala Gly  Phe Asp Ala Trp Phe  Ser Gln Arg Gly Gly  Ser Tyr Lys
    2810                 2815                 2820

Asn Asp  Lys Ser Cys Pro Val  Val Ala Ala Ile Ile  Thr Arg Glu
    2825                 2830                 2835

Ile Gly  Phe Ile Val Pro Gly  Leu Pro Gly Thr Val  Leu Arg Ala
    2840                 2845                 2850

Ile Asn  Gly Asp Phe Leu His  Phe Leu Pro Arg Val  Phe Ser Ala
    2855                 2860                 2865

Val Gly  Asn Ile Cys Tyr Thr  Pro Ser Lys Leu Ile  Glu Tyr Ser
    2870                 2875                 2880

Asp Phe  Ala Thr Ser Ala Cys  Val Leu Ala Ala Glu  Cys Thr Ile
    2885                 2890                 2895

Phe Lys  Asp Ala Met Gly Lys  Pro Val Pro Tyr Cys  Tyr Asp Thr
    2900                 2905                 2910

Asn Leu  Leu Glu Gly Ser Ile  Ser Tyr Ser Glu Leu  Arg Pro Asp
    2915                 2920                 2925
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Thr Arg  Tyr Val Leu Met Asp  Gly Ser Ile Ile Gln  Phe Pro Asn
    2930                 2935                 2940

Thr Tyr  Leu Glu Gly Ser Val  Arg Val Val Thr Thr  Phe Asp Ala
    2945                 2950                 2955

Glu Tyr  Cys Arg His Gly Thr  Cys Glu Arg Ser Glu  Val Gly Ile
    2960                 2965                 2970

Cys Leu  Ser Thr Ser Gly Arg  Trp Val Leu Asn Asn  Glu His Tyr
    2975                 2980                 2985

Arg Ala  Leu Ser Gly Val Phe  Cys Gly Val Asp Ala  Met Asn Leu
    2990                 2995                 3000

Ile Ala  Asn Ile Phe Thr Pro  Leu Val Gln Pro Val  Gly Ala Leu
    3005                 3010                 3015

Asp Val  Ser Ala Ser Val Val  Ala Gly Gly Ile Ile  Ala Ile Leu
    3020                 3025                 3030

Val Thr  Cys Ala Ala Tyr Tyr  Phe Met Lys Phe Arg  Arg Val Phe
    3035                 3040                 3045

Gly Glu  Tyr Asn His Val Val  Ala Ala Asn Ala Leu  Leu Phe Leu
    3050                 3055                 3060

Met Ser  Phe Thr Ile Leu Cys  Leu Val Pro Ala Tyr  Ser Phe Leu
    3065                 3070                 3075

Pro Gly  Val Tyr Ser Val Phe  Tyr Leu Tyr Leu Thr  Phe Tyr Phe
    3080                 3085                 3090

Thr Asn  Asp Val Ser Phe Leu  Ala His Leu Gln Trp  Phe Ala Met
    3095                 3100                 3105

Phe Ser  Pro Ile Val Pro Phe  Trp Ile Thr Ala Ile  Tyr Val Phe
    3110                 3115                 3120

Cys Ile  Ser Leu Lys His Cys  His Trp Phe Phe Asn  Asn Tyr Leu
    3125                 3130                 3135

Arg Lys  Arg Val Met Phe Asn  Gly Val Thr Phe Ser  Thr Phe Glu
    3140                 3145                 3150

Glu Ala  Ala Leu Cys Thr Phe  Leu Leu Asn Lys Glu  Met Tyr Leu
    3155                 3160                 3165

Lys Leu  Arg Ser Glu Thr Leu  Leu Pro Leu Thr Gln  Tyr Asn Arg
    3170                 3175                 3180

Tyr Leu  Ala Leu Tyr Asn Lys  Tyr Lys Tyr Phe Ser  Gly Ala Leu
    3185                 3190                 3195

Asp Thr  Thr Ser Tyr Arg Glu  Ala Ala Cys Cys His  Leu Ala Lys
    3200                 3205                 3210

Ala Leu  Asn Asp Phe Ser Asn  Ser Gly Ala Asp Val  Leu Tyr Gln
    3215                 3220                 3225

Pro Pro  Gln Thr Ser Ile Thr  Ser Ala Val Leu Gln  Ser Gly Phe
    3230                 3235                 3240

Arg Lys  Met Ala Phe Pro Ser  Gly Lys Val Glu Gly  Cys Met Val
    3245                 3250                 3255

Gln Val  Thr Cys Gly Thr Thr  Thr Leu Asn Gly Leu  Trp Leu Asp
    3260                 3265                 3270

Asp Thr  Val Tyr Cys Pro Arg  His Val Ile Cys Thr  Ala Glu Asp
    3275                 3280                 3285

Met Leu  Asn Pro Asn Tyr Glu  Asp Leu Leu Ile Arg  Lys Ser Asn
    3290                 3295                 3300

His Ser  Phe Leu Val Gln Ala  Gly Asn Val Gln Leu  Arg Val Ile
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    3305                3310                3315

Gly His  Ser Met Gln Asn Cys  Leu Leu Arg Leu Lys  Val Asp Thr
    3320                3325                3330

Ser Asn  Pro Lys Thr Pro Lys  Tyr Lys Phe Val Arg  Ile Gln Pro
    3335                3340                3345

Gly Gln  Thr Phe Ser Val Leu  Ala Cys Tyr Asn Gly  Ser Pro Ser
    3350                3355                3360

Gly Val  Tyr Gln Cys Ala Met  Arg Pro Asn His Thr  Ile Lys Gly
    3365                3370                3375

Ser Phe  Leu Asn Gly Ser Cys  Gly Ser Val Gly Phe  Asn Ile Asp
    3380                3385                3390

Tyr Asp  Cys Val Ser Phe Cys  Tyr Met His His Met  Glu Leu Pro
    3395                3400                3405

Thr Gly  Val His Ala Gly Thr  Asp Leu Glu Gly Lys  Phe Tyr Gly
    3410                3415                3420

Pro Phe  Val Asp Arg Gln Thr  Ala Gln Ala Ala Gly  Thr Asp Thr
    3425                3430                3435

Thr Ile  Thr Leu Asn Val Leu  Ala Trp Leu Tyr Ala  Ala Val Ile
    3440                3445                3450

Asn Gly  Asp Arg Trp Phe Leu  Asn Arg Phe Thr Thr  Thr Leu Asn
    3455                3460                3465

Asp Phe  Asn Leu Val Ala Met  Lys Tyr Asn Tyr Glu  Pro Leu Thr
    3470                3475                3480

Gln Asp  His Val Asp Ile Leu  Gly Pro Leu Ser Ala  Gln Thr Gly
    3485                3490                3495

Ile Ala  Val Leu Asp Met Cys  Ala Ala Leu Lys Glu  Leu Leu Gln
    3500                3505                3510

Asn Gly  Met Asn Gly Arg Thr  Ile Leu Gly Ser Thr  Ile Leu Glu
    3515                3520                3525

Asp Glu  Phe Thr Pro Phe Asp  Val Val Arg Gln Cys  Ser Gly Val
    3530                3535                3540

Thr Phe  Gln Gly Lys Phe Lys  Lys Ile Val Lys Gly  Thr His His
    3545                3550                3555

Trp Met  Leu Leu Thr Phe Leu  Thr Ser Leu Leu Ile  Leu Val Gln
    3560                3565                3570

Ser Thr  Gln Trp Ser Leu Phe  Phe Phe Val Tyr Glu  Asn Ala Phe
    3575                3580                3585

Leu Pro  Phe Thr Leu Gly Ile  Met Ala Ile Ala Ala  Cys Ala Met
    3590                3595                3600

Leu Leu  Val Lys His Lys His  Ala Phe Leu Cys Leu  Phe Leu Leu
    3605                3610                3615

Pro Ser  Leu Ala Thr Val Ala  Tyr Phe Asn Met Val  Tyr Met Pro
    3620                3625                3630

Ala Ser  Trp Val Met Arg Ile  Met Thr Trp Leu Glu  Leu Ala Asp
    3635                3640                3645

Thr Ser  Leu Ser Gly Tyr Arg  Leu Lys Asp Cys Val  Met Tyr Ala
    3650                3655                3660

Ser Ala  Leu Val Leu Leu Ile  Leu Met Thr Ala Arg  Thr Val Tyr
    3665                3670                3675

Asp Asp  Ala Ala Arg Arg Val  Trp Thr Leu Met Asn  Val Ile Thr
    3680                3685                3690
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Leu Val  Tyr Lys Val Tyr Tyr  Gly Asn Ala Leu Asp  Gln Ala Ile
    3695                 3700                 3705

Ser Met  Trp Ala Leu Val Ile  Ser Val Thr Ser Asn  Tyr Ser Gly
    3710                 3715                 3720

Val Val  Thr Thr Ile Met Phe  Leu Ala Arg Ala Ile  Val Phe Val
    3725                 3730                 3735

Cys Val  Glu Tyr Tyr Pro Leu  Leu Phe Ile Thr Gly  Asn Thr Leu
    3740                 3745                 3750

Gln Cys  Ile Met Leu Val Tyr  Cys Phe Leu Gly Tyr  Cys Cys Cys
    3755                 3760                 3765

Cys Tyr  Phe Gly Leu Phe Cys  Leu Leu Asn Arg Tyr  Phe Arg Leu
    3770                 3775                 3780

Thr Leu  Gly Val Tyr Asp Tyr  Leu Val Ser Thr Gln  Glu Phe Arg
    3785                 3790                 3795

Tyr Met  Asn Ser Gln Gly Leu  Leu Pro Pro Lys Ser  Ser Ile Asp
    3800                 3805                 3810

Ala Phe  Lys Leu Asn Ile Lys  Leu Leu Gly Ile Gly  Gly Lys Pro
    3815                 3820                 3825

Cys Ile  Lys Val Ala Thr Val  Gln Ser Lys Met Ser  Asp Val Lys
    3830                 3835                 3840

Cys Thr  Ser Val Val Leu Leu  Ser Val Leu Gln Gln  Leu Arg Val
    3845                 3850                 3855

Glu Ser  Ser Ser Lys Leu Trp  Ala Gln Cys Val Gln  Leu His Asn
    3860                 3865                 3870

Asp Ile  Leu Leu Ala Lys Asp  Thr Thr Glu Ala Phe  Glu Lys Met
    3875                 3880                 3885

Val Ser  Leu Leu Ser Val Leu  Leu Ser Met Gln Gly  Ala Val Asp
    3890                 3895                 3900

Ile Asn  Arg Leu Cys Glu Glu  Met Leu Asp Asn Arg  Ala Thr Leu
    3905                 3910                 3915

Gln Ala  Ile Ala Ser Glu Phe  Ser Ser Leu Pro Ser  Tyr Ala Ala
    3920                 3925                 3930

Tyr Ala  Thr Ala Gln Glu Ala  Tyr Glu Gln Ala Val  Ala Asn Gly
    3935                 3940                 3945

Asp Ser  Glu Val Val Leu Lys  Lys Leu Lys Lys Ser  Leu Asn Val
    3950                 3955                 3960

Ala Lys  Ser Glu Phe Asp Arg  Asp Ala Ala Met Gln  Arg Lys Leu
    3965                 3970                 3975

Glu Lys  Met Ala Asp Gln Ala  Met Thr Gln Met Tyr  Lys Gln Ala
    3980                 3985                 3990

Arg Ser  Glu Asp Lys Arg Ala  Lys Val Thr Ser Ala  Met Gln Thr
    3995                 4000                 4005

Met Leu  Phe Thr Met Leu Arg  Lys Leu Asp Asn Asp  Ala Leu Asn
    4010                 4015                 4020

Asn Ile  Ile Asn Asn Ala Arg  Asp Gly Cys Val Pro  Leu Asn Ile
    4025                 4030                 4035

Ile Pro  Leu Thr Thr Ala Ala  Lys Leu Met Val Val  Val Pro Asp
    4040                 4045                 4050

Tyr Gly  Thr Tyr Lys Asn Thr  Cys Asp Gly Asn Thr  Phe Thr Tyr
    4055                 4060                 4065
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Ala Ser  Ala Leu Trp Glu Ile  Gln Gln Val Val Asp  Ala Asp Ser
    4070                 4075                 4080

Lys Ile  Val Gln Leu Ser Glu  Ile Asn Met Asp Asn  Ser Pro Asn
    4085                 4090                 4095

Leu Ala  Trp Pro Leu Ile Val  Thr Ala Leu Arg Ala  Asn Ser Ala
    4100                 4105                 4110

Val Lys  Leu Gln Asn Asn Glu  Leu Ser Pro Val Ala  Leu Arg Gln
    4115                 4120                 4125

Met Ser  Cys Ala Ala Gly Thr  Thr Gln Thr Ala Cys  Thr Asp Asp
    4130                 4135                 4140

Asn Ala  Leu Ala Tyr Tyr Asn  Asn Ser Lys Gly Gly  Arg Phe Val
    4145                 4150                 4155

Leu Ala  Leu Leu Ser Asp His  Gln Asp Leu Lys Trp  Ala Arg Phe
    4160                 4165                 4170

Pro Lys  Ser Asp Gly Thr Gly  Thr Ile Tyr Thr Glu  Leu Glu Pro
    4175                 4180                 4185

Pro Cys  Arg Phe Val Thr Asp  Thr Pro Lys Gly Pro  Lys Val Lys
    4190                 4195                 4200

Tyr Leu  Tyr Phe Ile Lys Gly  Leu Asn Asn Leu Asn  Arg Gly Met
    4205                 4210                 4215

Val Leu  Gly Ser Leu Ala Ala  Thr Val Arg Leu Gln  Ala Gly Asn
    4220                 4225                 4230

Ala Thr  Glu Val Pro Ala Asn  Ser Thr Val Leu Ser  Phe Cys Ala
    4235                 4240                 4245

Phe Ala  Val Asp Pro Ala Lys  Ala Tyr Lys Asp Tyr  Leu Ala Ser
    4250                 4255                 4260

Gly Gly  Gln Pro Ile Thr Asn  Cys Val Lys Met Leu  Cys Thr His
    4265                 4270                 4275

Thr Gly  Thr Gly Gln Ala Ile  Thr Val Thr Pro Glu  Ala Asn Met
    4280                 4285                 4290

Asp Gln  Glu Ser Phe Gly Gly  Ala Ser Cys Cys Leu  Tyr Cys Arg
    4295                 4300                 4305

Cys His  Ile Asp His Pro Asn  Pro Lys Gly Phe Cys  Asp Leu Lys
    4310                 4315                 4320

Gly Lys  Tyr Val Gln Ile Pro  Thr Thr Cys Ala Asn  Asp Pro Val
    4325                 4330                 4335

Gly Phe  Thr Leu Arg Asn Thr  Val Cys Thr Val Cys  Gly Met Trp
    4340                 4345                 4350

Lys Gly  Tyr Gly Cys Ser Cys  Asp Gln Leu Arg Glu  Pro Leu Met
    4355                 4360                 4365

Gln Ser  Ala Asp Ala Ser Thr  Phe Leu Asn Gly Phe  Ala Val
    4370                 4375                 4380


<210> SEQ ID NO 75
<211> LENGTH: 2695
<212> TYPE: PRT
<213> ORGANISM: CORONAVIRUS

<400> SEQUENCE: 75

Arg Val Cys Gly Val Ser Ala Ala Arg Leu Thr Pro Cys Gly Thr Gly
1               5                  10                  15

Thr Ser Thr Asp Val Val Tyr Arg Ala Phe Asp Ile Tyr Asn Glu Lys
            20                  25                  30
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Val Ala Gly Phe Ala Lys Phe Leu Lys Thr Asn Cys Cys Arg Phe Gln
        35                  40                  45

Glu Lys Asp Glu Glu Gly Asn Leu Leu Asp Ser Tyr Phe Val Val Lys
    50                  55                  60

Arg His Thr Met Ser Asn Tyr Gln His Glu Glu Thr Ile Tyr Asn Leu
65                  70                  75                  80

Val Lys Asp Cys Pro Ala Val Ala Val His Asp Phe Phe Lys Phe Arg
                85                  90                  95

Val Asp Gly Asp Met Val Pro His Ile Ser Arg Gln Arg Leu Thr Lys
            100                 105                 110

Tyr Thr Met Ala Asp Leu Val Tyr Ala Leu Arg His Phe Asp Glu Gly
        115                 120                 125

Asn Cys Asp Thr Leu Lys Glu Ile Leu Val Thr Tyr Asn Cys Cys Asp
    130                 135                 140

Asp Asp Tyr Phe Asn Lys Lys Asp Trp Tyr Asp Phe Val Glu Asn Pro
145                 150                 155                 160

Asp Ile Leu Arg Val Tyr Ala Asn Leu Gly Glu Arg Val Arg Gln Ser
                165                 170                 175

Leu Leu Lys Thr Val Gln Phe Cys Asp Ala Met Arg Asp Ala Gly Ile
            180                 185                 190

Val Gly Val Leu Thr Leu Asp Asn Gln Asp Leu Asn Gly Asn Trp Tyr
        195                 200                 205

Asp Phe Gly Asp Phe Val Gln Val Ala Pro Gly Cys Gly Val Pro Ile
    210                 215                 220

Val Asp Ser Tyr Tyr Ser Leu Leu Met Pro Ile Leu Thr Leu Thr Arg
225                 230                 235                 240

Ala Leu Ala Ala Glu Ser His Met Asp Ala Asp Leu Ala Lys Pro Leu
                245                 250                 255

Ile Lys Trp Asp Leu Leu Lys Tyr Asp Phe Thr Glu Glu Arg Leu Cys
            260                 265                 270

Leu Phe Asp Arg Tyr Phe Lys Tyr Trp Asp Gln Thr Tyr His Pro Asn
        275                 280                 285

Cys Ile Asn Cys Leu Asp Asp Arg Cys Ile Leu His Cys Ala Asn Phe
    290                 295                 300

Asn Val Leu Phe Ser Thr Val Phe Pro Pro Thr Ser Phe Gly Pro Leu
305                 310                 315                 320

Val Arg Lys Ile Phe Val Asp Gly Val Pro Phe Val Val Ser Thr Gly
                325                 330                 335

Tyr His Phe Arg Glu Leu Gly Val Val His Asn Gln Asp Val Asn Leu
            340                 345                 350

His Ser Ser Arg Leu Ser Phe Lys Glu Leu Leu Val Tyr Ala Ala Asp
        355                 360                 365

Pro Ala Met His Ala Ala Ser Gly Asn Leu Leu Leu Asp Lys Arg Thr
    370                 375                 380

Thr Cys Phe Ser Val Ala Ala Leu Thr Asn Asn Val Ala Phe Gln Thr
385                 390                 395                 400

Val Lys Pro Gly Asn Phe Asn Lys Asp Phe Tyr Asp Phe Ala Val Ser
                405                 410                 415

Lys Gly Phe Phe Lys Glu Gly Ser Ser Val Glu Leu Lys His Phe Phe
            420                 425                 430
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Phe Ala Gln Asp Gly Asn Ala Ala Ile Ser Asp Tyr Asp Tyr Tyr Arg
        435                 440                 445

Tyr Asn Leu Pro Thr Met Cys Asp Ile Arg Gln Leu Leu Phe Val Val
    450                 455                 460

Glu Val Val Asp Lys Tyr Phe Asp Cys Tyr Asp Gly Gly Cys Ile Asn
465                 470                 475                 480

Ala Asn Gln Val Ile Val Asn Asn Leu Asp Lys Ser Ala Gly Phe Pro
                485                 490                 495

Phe Asn Lys Trp Gly Lys Ala Arg Leu Tyr Tyr Asp Ser Met Ser Tyr
            500                 505                 510

Glu Asp Gln Asp Ala Leu Phe Ala Tyr Thr Lys Arg Asn Val Ile Pro
        515                 520                 525

Thr Ile Thr Gln Met Asn Leu Lys Tyr Ala Ile Ser Ala Lys Asn Arg
    530                 535                 540

Ala Arg Thr Val Ala Gly Val Ser Ile Cys Ser Thr Met Thr Asn Arg
545                 550                 555                 560

Gln Phe His Gln Lys Leu Leu Lys Ser Ile Ala Ala Thr Arg Gly Ala
                565                 570                 575

Thr Val Val Ile Gly Thr Ser Lys Phe Tyr Gly Gly Trp His Asn Met
            580                 585                 590

Leu Lys Thr Val Tyr Ser Asp Val Glu Thr Pro His Leu Met Gly Trp
        595                 600                 605

Asp Tyr Pro Lys Cys Asp Arg Ala Met Pro Asn Met Leu Arg Ile Met
    610                 615                 620

Ala Ser Leu Val Leu Ala Arg Lys His Asn Thr Cys Cys Asn Leu Ser
625                 630                 635                 640

His Arg Phe Tyr Arg Leu Ala Asn Glu Cys Ala Gln Val Leu Ser Glu
                645                 650                 655

Met Val Met Cys Gly Gly Ser Leu Tyr Val Lys Pro Gly Gly Thr Ser
            660                 665                 670

Ser Gly Asp Ala Thr Thr Ala Tyr Ala Asn Ser Val Phe Asn Ile Cys
        675                 680                 685

Gln Ala Val Thr Ala Asn Val Asn Ala Leu Leu Ser Thr Asp Gly Asn
    690                 695                 700

Lys Ile Ala Asp Lys Tyr Val Arg Asn Leu Gln His Arg Leu Tyr Glu
705                 710                 715                 720

Cys Leu Tyr Arg Asn Arg Asp Val Asp His Glu Phe Val Asp Glu Phe
                725                 730                 735

Tyr Ala Tyr Leu Arg Lys His Phe Ser Met Met Ile Leu Ser Asp Asp
            740                 745                 750

Ala Val Val Cys Tyr Asn Ser Asn Tyr Ala Ala Gln Gly Leu Val Ala
        755                 760                 765

Ser Ile Lys Asn Phe Lys Ala Val Leu Tyr Tyr Gln Asn Asn Val Phe
    770                 775                 780

Met Ser Glu Ala Lys Cys Trp Thr Glu Thr Asp Leu Thr Lys Gly Pro
785                 790                 795                 800

His Glu Phe Cys Ser Gln His Thr Met Leu Val Lys Gln Gly Asp Asp
                805                 810                 815

Tyr Val Tyr Leu Pro Tyr Pro Asp Pro Ser Arg Ile Leu Gly Ala Gly
            820                 825                 830

Cys Phe Val Asp Asp Ile Val Lys Thr Asp Gly Thr Leu Met Ile Glu
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        835                 840                 845

Arg Phe Val Ser Leu Ala Ile Asp Ala Tyr Pro Leu Thr Lys His Pro
    850                 855                 860

Asn Gln Glu Tyr Ala Asp Val Phe His Leu Tyr Leu Gln Tyr Ile Arg
865                 870                 875                 880

Lys Leu His Asp Glu Leu Thr Gly His Met Leu Asp Met Tyr Ser Val
                885                 890                 895

Met Leu Thr Asn Asp Asn Thr Ser Arg Tyr Trp Glu Pro Glu Phe Tyr
            900                 905                 910

Glu Ala Met Tyr Thr Pro His Thr Val Leu Gln Ala Val Gly Ala Cys
        915                 920                 925

Val Leu Cys Asn Ser Gln Thr Ser Leu Arg Cys Gly Ala Cys Ile Arg
    930                 935                 940

Arg Pro Phe Leu Cys Cys Lys Cys Cys Tyr Asp His Val Ile Ser Thr
945                 950                 955                 960

Ser His Lys Leu Val Leu Ser Val Asn Pro Tyr Val Cys Asn Ala Pro
                965                 970                 975

Gly Cys Asp Val Thr Asp Val Thr Gln Leu Tyr Leu Gly Gly Met Ser
            980                 985                 990

Tyr Tyr Cys Lys Ser His Lys Pro Pro Ile Ser Phe Pro Leu Cys Ala
        995                 1000                1005

Asn Gly  Gln Val Phe Gly Leu  Tyr Lys Asn Thr Cys  Val Gly Ser
    1010                1015                1020

Asp Asn  Val Thr Asp Phe Asn  Ala Ile Ala Thr Cys  Asp Trp Thr
    1025                1030                1035

Asn Ala  Gly Asp Tyr Ile Leu  Ala Asn Thr Cys Thr  Glu Arg Leu
    1040                1045                1050

Lys Leu  Phe Ala Ala Glu Thr  Leu Lys Ala Thr Glu  Glu Thr Phe
    1055                1060                1065

Lys Leu  Ser Tyr Gly Ile Ala  Thr Val Arg Glu Val  Leu Ser Asp
    1070                1075                1080

Arg Glu  Leu His Leu Ser Trp  Glu Val Gly Lys Pro  Arg Pro Pro
    1085                1090                1095

Leu Asn  Arg Asn Tyr Val Phe  Thr Gly Tyr Arg Val  Thr Lys Asn
    1100                1105                1110

Ser Lys  Val Gln Ile Gly Glu  Tyr Thr Phe Glu Lys  Gly Asp Tyr
    1115                1120                1125

Gly Asp  Ala Val Val Tyr Arg  Gly Thr Thr Thr Tyr  Lys Leu Asn
    1130                1135                1140

Val Gly  Asp Tyr Phe Val Leu  Thr Ser His Thr Val  Met Pro Leu
    1145                1150                1155

Ser Ala  Pro Thr Leu Val Pro  Gln Glu His Tyr Val  Arg Ile Thr
    1160                1165                1170

Gly Leu  Tyr Pro Thr Leu Asn  Ile Ser Asp Glu Phe  Ser Ser Asn
    1175                1180                1185

Val Ala  Asn Tyr Gln Lys Val  Gly Met Gln Lys Tyr  Ser Thr Leu
    1190                1195                1200

Gln Gly  Pro Pro Gly Thr Gly  Lys Ser His Phe Ala  Ile Gly Leu
    1205                1210                1215

Ala Leu  Tyr Tyr Pro Ser Ala  Arg Ile Val Tyr Thr  Ala Cys Ser
    1220                1225                1230
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His Ala  Ala Val Asp Ala Leu  Cys Glu Lys Ala Leu  Lys Tyr Leu
    1235                 1240                 1245

Pro Ile  Asp Lys Cys Ser Arg  Ile Ile Pro Ala Arg  Ala Arg Val
    1250                 1255                 1260

Glu Cys  Phe Asp Lys Phe Lys  Val Asn Ser Thr Leu  Glu Gln Tyr
    1265                 1270                 1275

Val Phe  Cys Thr Val Asn Ala  Leu Pro Glu Thr Thr  Ala Asp Ile
    1280                 1285                 1290

Val Val  Phe Asp Glu Ile Ser  Met Ala Thr Asn Tyr  Asp Leu Ser
    1295                 1300                 1305

Val Val  Asn Ala Arg Leu Arg  Ala Lys His Tyr Val  Tyr Ile Gly
    1310                 1315                 1320

Asp Pro  Ala Gln Leu Pro Ala  Pro Arg Thr Leu Leu  Thr Lys Gly
    1325                 1330                 1335

Thr Leu  Glu Pro Glu Tyr Phe  Asn Ser Val Cys Arg  Leu Met Lys
    1340                 1345                 1350

Thr Ile  Gly Pro Asp Met Phe  Leu Gly Thr Cys Arg  Arg Cys Pro
    1355                 1360                 1365

Ala Glu  Ile Val Asp Thr Val  Ser Ala Leu Val Tyr  Asp Asn Lys
    1370                 1375                 1380

Leu Lys  Ala His Lys Asp Lys  Ser Ala Gln Cys Phe  Lys Met Phe
    1385                 1390                 1395

Tyr Lys  Gly Val Ile Thr His  Asp Val Ser Ser Ala  Ile Asn Arg
    1400                 1405                 1410

Pro Gln  Ile Gly Val Val Arg  Glu Phe Leu Thr Arg  Asn Pro Ala
    1415                 1420                 1425

Trp Arg  Lys Ala Val Phe Ile  Ser Pro Tyr Asn Ser  Gln Asn Ala
    1430                 1435                 1440

Val Ala  Ser Lys Ile Leu Gly  Leu Pro Thr Gln Thr  Val Asp Ser
    1445                 1450                 1455

Ser Gln  Gly Ser Glu Tyr Asp  Tyr Val Ile Phe Thr  Gln Thr Thr
    1460                 1465                 1470

Glu Thr  Ala His Ser Cys Asn  Val Asn Arg Phe Asn  Val Ala Ile
    1475                 1480                 1485

Thr Arg  Ala Lys Ile Gly Ile  Leu Cys Ile Met Ser  Asp Arg Asp
    1490                 1495                 1500

Leu Tyr  Asp Lys Leu Gln Phe  Thr Ser Leu Glu Ile  Pro Arg Arg
    1505                 1510                 1515

Asn Val  Ala Thr Leu Gln Ala  Glu Asn Val Thr Gly  Leu Phe Lys
    1520                 1525                 1530

Asp Cys  Ser Lys Ile Ile Thr  Gly Leu His Pro Thr  Gln Ala Pro
    1535                 1540                 1545

Thr His  Leu Ser Val Asp Ile  Lys Phe Lys Thr Glu  Gly Leu Cys
    1550                 1555                 1560

Val Asp  Ile Pro Gly Ile Pro  Lys Asp Met Thr Tyr  Arg Arg Leu
    1565                 1570                 1575

Ile Ser  Met Met Gly Phe Lys  Met Asn Tyr Gln Val  Asn Gly Tyr
    1580                 1585                 1590

Pro Asn  Met Phe Ile Thr Arg  Glu Glu Ala Ile Arg  His Val Arg
    1595                 1600                 1605
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Ala Trp  Ile Gly Phe Asp Val  Glu Gly Cys His Ala  Thr Arg Asp
    1610                 1615                 1620

Ala Val  Gly Thr Asn Leu Pro  Leu Gln Leu Gly Phe  Ser Thr Gly
    1625                 1630                 1635

Val Asn  Leu Val Ala Val Pro  Thr Gly Tyr Val Asp  Thr Glu Asn
    1640                 1645                 1650

Asn Thr  Glu Phe Thr Arg Val  Asn Ala Lys Pro Pro  Pro Gly Asp
    1655                 1660                 1665

Gln Phe  Lys His Leu Ile Pro  Leu Met Tyr Lys Gly  Leu Pro Trp
    1670                 1675                 1680

Asn Val  Val Arg Ile Lys Ile  Val Gln Met Leu Ser  Asp Thr Leu
    1685                 1690                 1695

Lys Gly  Leu Ser Asp Arg Val  Val Phe Val Leu Trp  Ala His Gly
    1700                 1705                 1710

Phe Glu  Leu Thr Ser Met Lys  Tyr Phe Val Lys Ile  Gly Pro Glu
    1715                 1720                 1725

Arg Thr  Cys Cys Leu Cys Asp  Lys Arg Ala Thr Cys  Phe Ser Thr
    1730                 1735                 1740

Ser Ser  Asp Thr Tyr Ala Cys  Trp Asn His Ser Val  Gly Phe Asp
    1745                 1750                 1755

Tyr Val  Tyr Asn Pro Phe Met  Ile Asp Val Gln Gln  Trp Gly Phe
    1760                 1765                 1770

Thr Gly  Asn Leu Gln Ser Asn  His Asp Gln His Cys  Gln Val His
    1775                 1780                 1785

Gly Asn  Ala His Val Ala Ser  Cys Asp Ala Ile Met  Thr Arg Cys
    1790                 1795                 1800

Leu Ala  Val His Glu Cys Phe  Val Lys Arg Val Asp  Trp Ser Val
    1805                 1810                 1815

Glu Tyr  Pro Ile Ile Gly Asp  Glu Leu Arg Val Asn  Ser Ala Cys
    1820                 1825                 1830

Arg Lys  Val Gln His Met Val  Val Lys Ser Ala Leu  Leu Ala Asp
    1835                 1840                 1845

Lys Phe  Pro Val Leu His Asp  Ile Gly Asn Pro Lys  Ala Ile Lys
    1850                 1855                 1860

Cys Val  Pro Gln Ala Glu Val  Glu Trp Lys Phe Tyr  Asp Ala Gln
    1865                 1870                 1875

Pro Cys  Ser Asp Lys Ala Tyr  Lys Ile Glu Glu Leu  Phe Tyr Ser
    1880                 1885                 1890

Tyr Ala  Thr His His Asp Lys  Phe Thr Asp Gly Val  Cys Leu Phe
    1895                 1900                 1905

Trp Asn  Cys Asn Val Asp Arg  Tyr Pro Ala Asn Ala  Ile Val Cys
    1910                 1915                 1920

Arg Phe  Asp Thr Arg Val Leu  Ser Asn Leu Asn Leu  Pro Gly Cys
    1925                 1930                 1935

Asp Gly  Gly Ser Leu Tyr Val  Asn Lys His Ala Phe  His Thr Pro
    1940                 1945                 1950

Ala Phe  Asp Lys Ser Ala Phe  Thr Asn Leu Lys Gln  Leu Pro Phe
    1955                 1960                 1965

Phe Tyr  Tyr Ser Asp Ser Pro  Cys Glu Ser His Gly  Lys Gln Val
    1970                 1975                 1980

Val Ser  Asp Ile Asp Tyr Val  Pro Leu Lys Ser Ala  Thr Cys Ile
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    1985                1990                1995

Thr Arg  Cys Asn Leu Gly Gly  Ala Val Cys Arg His  His Ala Asn
    2000                2005                2010

Glu Tyr  Arg Gln Tyr Leu Asp  Ala Tyr Asn Met Met  Ile Ser Ala
    2015                2020                2025

Gly Phe  Ser Leu Trp Ile Tyr  Lys Gln Phe Asp Thr  Tyr Asn Leu
    2030                2035                2040

Trp Asn  Thr Phe Thr Arg Leu  Gln Ser Leu Glu Asn  Val Ala Tyr
    2045                2050                2055

Asn Val  Val Asn Lys Gly His  Phe Asp Gly His Ala  Gly Glu Ala
    2060                2065                2070

Pro Val  Ser Ile Ile Asn Asn  Ala Val Tyr Thr Lys  Val Asp Gly
    2075                2080                2085

Ile Asp  Val Glu Ile Phe Glu  Asn Lys Thr Thr Leu  Pro Val Asn
    2090                2095                2100

Val Ala  Phe Glu Leu Trp Ala  Lys Arg Asn Ile Lys  Pro Val Pro
    2105                2110                2115

Glu Ile  Lys Ile Leu Asn Asn  Leu Gly Val Asp Ile  Ala Ala Asn
    2120                2125                2130

Thr Val  Ile Trp Asp Tyr Lys  Arg Glu Ala Pro Ala  His Val Ser
    2135                2140                2145

Thr Ile  Gly Val Cys Thr Met  Thr Asp Ile Ala Lys  Lys Pro Thr
    2150                2155                2160

Glu Ser  Ala Cys Ser Ser Leu  Thr Val Leu Phe Asp  Gly Arg Val
    2165                2170                2175

Glu Gly  Gln Val Asp Leu Phe  Arg Asn Ala Arg Asn  Gly Val Leu
    2180                2185                2190

Ile Thr  Glu Gly Ser Val Lys  Gly Leu Thr Pro Ser  Lys Gly Pro
    2195                2200                2205

Ala Gln  Ala Ser Val Asn Gly  Val Thr Leu Ile Gly  Glu Ser Val
    2210                2215                2220

Lys Thr  Gln Phe Asn Tyr Phe  Lys Lys Val Asp Gly  Ile Ile Gln
    2225                2230                2235

Gln Leu  Pro Glu Thr Tyr Phe  Thr Gln Ser Arg Asp  Leu Glu Asp
    2240                2245                2250

Phe Lys  Pro Arg Ser Gln Met  Glu Thr Asp Phe Leu  Glu Leu Ala
    2255                2260                2265

Met Asp  Glu Phe Ile Gln Arg  Tyr Lys Leu Glu Gly  Tyr Ala Phe
    2270                2275                2280

Glu His  Ile Val Tyr Gly Asp  Phe Ser His Gly Gln  Leu Gly Gly
    2285                2290                2295

Leu His  Leu Met Ile Gly Leu  Ala Lys Arg Ser Gln  Asp Ser Pro
    2300                2305                2310

Leu Lys  Leu Glu Asp Phe Ile  Pro Met Asp Ser Thr  Val Lys Asn
    2315                2320                2325

Tyr Phe  Ile Thr Asp Ala Gln  Thr Gly Ser Ser Lys  Cys Val Cys
    2330                2335                2340

Ser Val  Ile Asp Leu Leu Leu  Asp Asp Phe Val Glu  Ile Ile Lys
    2345                2350                2355

Ser Gln  Asp Leu Ser Val Ile  Ser Lys Val Val Lys  Val Thr Ile
    2360                2365                2370
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Asp Tyr  Ala Glu Ile Ser Phe  Met Leu Trp Cys Lys  Asp Gly His
    2375                 2380                 2385

Val Glu  Thr Phe Tyr Pro Lys  Leu Gln Ala Ser Gln  Ala Trp Gln
    2390                 2395                 2400

Pro Gly  Val Ala Met Pro Asn  Leu Tyr Lys Met Gln  Arg Met Leu
    2405                 2410                 2415

Leu Glu  Lys Cys Asp Leu Gln  Asn Tyr Gly Glu Asn  Ala Val Ile
    2420                 2425                 2430

Pro Lys  Gly Ile Met Met Asn  Val Ala Lys Tyr Thr  Gln Leu Cys
    2435                 2440                 2445

Gln Tyr  Leu Asn Thr Leu Thr  Leu Ala Val Pro Tyr  Asn Met Arg
    2450                 2455                 2460

Val Ile  His Phe Gly Ala Gly  Ser Asp Lys Gly Val  Ala Pro Gly
    2465                 2470                 2475

Thr Ala  Val Leu Arg Gln Trp  Leu Pro Thr Gly Thr  Leu Leu Val
    2480                 2485                 2490

Asp Ser  Asp Leu Asn Asp Phe  Val Ser Asp Ala Asp  Ser Thr Leu
    2495                 2500                 2505

Ile Gly  Asp Cys Ala Thr Val  His Thr Ala Asn Lys  Trp Asp Leu
    2510                 2515                 2520

Ile Ile  Ser Asp Met Tyr Asp  Pro Arg Thr Lys His  Val Thr Lys
    2525                 2530                 2535

Glu Asn  Asp Ser Lys Glu Gly  Phe Phe Thr Tyr Leu  Cys Gly Phe
    2540                 2545                 2550

Ile Lys  Gln Lys Leu Ala Leu  Gly Gly Ser Ile Ala  Val Lys Ile
    2555                 2560                 2565

Thr Glu  His Ser Trp Asn Ala  Asp Leu Tyr Lys Leu  Met Gly His
    2570                 2575                 2580

Phe Ser  Trp Trp Thr Ala Phe  Val Thr Asn Val Asn  Ala Ser Ser
    2585                 2590                 2595

Ser Glu  Ala Phe Leu Ile Gly  Ala Asn Tyr Leu Gly  Lys Pro Lys
    2600                 2605                 2610

Glu Gln  Ile Asp Gly Tyr Thr  Met His Ala Asn Tyr  Ile Phe Trp
    2615                 2620                 2625

Arg Asn  Thr Asn Pro Ile Gln  Leu Ser Ser Tyr Ser  Leu Phe Asp
    2630                 2635                 2640

Met Ser  Lys Phe Pro Leu Lys  Leu Arg Gly Thr Ala  Val Met Ser
    2645                 2650                 2655

Leu Lys  Glu Asn Gln Ile Asn  Asp Met Ile Tyr Ser  Leu Leu Glu
    2660                 2665                 2670

Lys Gly  Arg Leu Ile Ile Arg  Glu Asn Asn Arg Val  Val Val Ser
    2675                 2680                 2685

Ser Asp  Ile Leu Val Asn Asn
    2690                 2695


<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L3/+/4932 primer

<400> SEQUENCE: 76
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ccacacacag cttgtggata 20

<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/+/6401 primer

<400> SEQUENCE: 77

ccgaagttgt aggcaatgtc 20

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/+/6964 primer

<400> SEQUENCE: 78

tttggtgctc cttcttattg 20

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L4/-/6817 primer

<400> SEQUENCE: 79

ccggcatcca aacataattt 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/7633 primer

<400> SEQUENCE: 80

tggtcagtag ggttgattgg 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/8127 primer

<400> SEQUENCE: 81

catcctttgt gtcaacatcg 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/-/8633 primer

<400> SEQUENCE: 82

gtcacgagtg acaccatcct 20

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<210> SEQ ID NO 83
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/7839 primer

<400> SEQUENCE: 83

atgcgacgag tctgcttcta 20

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/8785 primer

<400> SEQUENCE: 84

ttcatagtgc ctggcttacc 20

<210> SEQ ID NO 85
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L5/+/8255 primer

<400> SEQUENCE: 85

atcttggcgc atgtattgac 20

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/9422 primer

<400> SEQUENCE: 86

tgcattagca gcaacaacat 20

<210> SEQ ID NO 87
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/9966 primer

<400> SEQUENCE: 87

tctgcagaac agcagaagtg 20

<210> SEQ ID NO 88
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/-/10542 primer

<400> SEQUENCE: 88

cctgtgcagt ttgtctgtca 20

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/10677 primer

<400> SEQUENCE: 89

ccttgtggca atgaagtaca 20

<210> SEQ ID NO 90
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/10106 primer

<400> SEQUENCE: 90

atgtcatttg cacagcagaa 20

<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L6/+/9571 primer

<400> SEQUENCE: 91

cttcaatggt ttgccatgtt 20

<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/11271 primer

<400> SEQUENCE: 92

tgcgagctgt catgagaata 20

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/11801 primer

<400> SEQUENCE: 93

aaccgagagc agtaccacag 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/-/12383 primer

<400> SEQUENCE: 94

tttggctgct gtagtcaatg 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/+/12640 primer

<400> SEQUENCE: 95

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ctacgacaga tgtcctgtgc 20

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/+/12088 primer

<400> SEQUENCE: 96

gagcaggctg tagctaatgg 20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L7/+/11551 primer

<400> SEQUENCE: 97

ttaggctatt gttgctgctg 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/-/13160 primer

<400> SEQUENCE: 98

cagacaacat gaagcaccac 20

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/-/13704 primer

<400> SEQUENCE: 99

cgctgacgtg atatatgtgg 20

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/-/14284 primer

<400> SEQUENCE: 100

tgcacaatga aggatacacc 20

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/+/14453 primer

<400> SEQUENCE: 101

acatagctcg cgtctcagtt 20

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<210> SEQ ID NO 102
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/+/13968 primer

<400> SEQUENCE: 102

ggcattgtag gcgtactgac 20

<210> SEQ ID NO 103
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L8/+/13401 primer

<400> SEQUENCE: 103

gtttgcggtg taagtgcag 19

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/15098 primer

<400> SEQUENCE: 104

tagtggcggc tattgacttc 20

<210> SEQ ID NO 105
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/15677 primer

<400> SEQUENCE: 105

ctaaaccttg agccgcatag 20

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/-/16247 primer

<400> SEQUENCE: 106

catggtcata gcagcacttg 20

<210> SEQ ID NO 107
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/16323 primer

<400> SEQUENCE: 107

ccaggttgtg atgtcactga t 21

<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/15858 primer

<400> SEQUENCE: 108

ccttacccag atccatcaag 20

<210> SEQ ID NO 109
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L9/+/15288 primer

<400> SEQUENCE: 109

cgcaaacata acacttgctg 20

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/-/16914 primer

<400> SEQUENCE: 110

agtgttgggt acaagccagt 20

<210> SEQ ID NO 111
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/-/17466 primer

<400> SEQUENCE: 111

gttccaagga acatgtctgg 20

<210> SEQ ID NO 112
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/-/18022 primer

<400> SEQUENCE: 112

aggtgcctgt gtaggatgaa 20

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/+/18245 primer

<400> SEQUENCE: 113

gggctgtcat gcaactagag 20

<210> SEQ ID NO 114
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/+/17663 primer

<400> SEQUENCE: 114

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tcttacacgc aatcctgctt 20

<210> SEQ ID NO 115
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L10/+/17061 primer

<400> SEQUENCE: 115

tacccatctg ctcgcatagt 20

<210> SEQ ID NO 116
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/-/18877 primer

<400> SEQUENCE: 116

gcaagcagaa ttaaccctca 20

<210> SEQ ID NO 117
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/-/19396 primer

<400> SEQUENCE: 117

agcaccacct aaattgcatc 20

<210> SEQ ID NO 118
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/-/20002 primer

<400> SEQUENCE: 118

tggtcccttt gaaggtgtta 20

<210> SEQ ID NO 119
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/20245 primer

<400> SEQUENCE: 119

tcgaacacat cgtttatgga 20

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/19611 primer

<400> SEQUENCE: 120

gaagcacctg tttccatcat 20

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<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: S/L11/+/19021 primer

<400> SEQUENCE: 121

acgatgctca gccatgtagt 20

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F3/+/800 primer

<400> SEQUENCE: 122

gaggtgcagt cactcgctat 20

<210> SEQ ID NO 123
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F4/+/1391 primer

<400> SEQUENCE: 123

cagagattgg acctgagcat 20

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/F5/+/1925 primer

<400> SEQUENCE: 124

cagcaaacca ctcaattcct 20

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/R3/-/1674 primer

<400> SEQUENCE: 125

aaatgatggc aacctcttca 20

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/R4/-/1107 primer

<400> SEQUENCE: 126

cacgtggttg aatgactttg 20

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: SARS/L1/R5/-/520 primer

<400> SEQUENCE: 127

atttctgcaa ccagctcaac 20

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/F3/+/2664 primer

<400> SEQUENCE: 128

cgcattgtct cctggtttac 20

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/F4/+/3232 primer

<400> SEQUENCE: 129

gagattgagc cagaaccaga 20

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/F5/+/3746 primer

<400> SEQUENCE: 130

atgagcaggt tgtcatggat 20

<210> SEQ ID NO 131
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/R3/-/3579 primer

<400> SEQUENCE: 131

ctgccttaag aagctggatg 20

<210> SEQ ID NO 132
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/R4/-/2991 primer

<400> SEQUENCE: 132

tttcttcacc agcatcatca 20

<210> SEQ ID NO 133
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L2/R5/-/2529 primer

<400> SEQUENCE: 133

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caccgttctt gagaacaacc 20

<210> SEQ ID NO 134
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/F3/+/4708 primer

<400> SEQUENCE: 134

tctttggctg gctcttacag 20

<210> SEQ ID NO 135
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SRAS/L3/F4/+/5305 primer

<400> SEQUENCE: 135

gctggtgatg ctgctaactt 20

<210> SEQ ID NO 136
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/F5/+/5822 primer

<400> SEQUENCE: 136

ccatcaagcc tgtgtcgtat 20

<210> SEQ ID NO 137
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<223> OTHER INFORMATION: SARS/L3/R3/-/5610 primer

<400> SEQUENCE: 137

caggtggtgc agacatcata 20

<210> SEQ ID NO 138
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: SARS/L3/R4/-/4988 primer

<400> SEQUENCE: 138

aacatcagca ccatccaagt 20

<210> SEQ ID NO 139
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 139

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<400> SEQUENCE: 140

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gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc 240

gcctggctga ccgcccaacg acccccgccc attgacgtca ataatgacgt atgttcccat 300

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cggtaaatgg cccgcctggc attatgccca gtacatgacc ttacgggact ttcctacttg 480

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caatgggcgt ggatagcggt ttgactcacg gggatttcca agtctccacc ccattgacgt 600

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gtgaggcact gggcaggtaa gtatcaaggt tacaagacag gtttaaggag accaatagaa 900

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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: SNE-S1 primer

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<212> TYPE: DNA
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<223> OTHER INFORMATION: SNE-AS1 primer

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<223> OTHER INFORMATION: SAR1-S primer

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<223> OTHER INFORMATION: PCR primer

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<210> SEQ ID NO 146
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<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 146

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<210> SEQ ID NO 147
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 147

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<210> SEQ ID NO 148
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 148

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<210> SEQ ID NO 149
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: N-terminal end of SRAS-CoV S protein
(amino acids 1 to 13)

<400> SEQUENCE: 149

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<400> SEQUENCE: 150

Ser Gly Asp Tyr Lys Asp Asp Asp Asp Lys
1 5 10

<210> SEQ ID NO 151
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<212> TYPE: DNA
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<400> SEQUENCE: 151

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<210> SEQ ID NO 152
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 154

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<210> SEQ ID NO 155
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
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<210> SEQ ID NO 156
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<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 156

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<210> SEQ ID NO 157
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 157

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<210> SEQ ID NO 158
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 158

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1. An isolated and purified protein or polypeptide, characterized in that it is the S protein having the sequence SEQ ID No: 3, its ectodomain or a fragment of its ectodomain.

2. The protein or polypeptide as claimed in claim 1, characterized in that it consists of the amino acids corresponding to positions 1 to 1193 of the amino acid sequence of the S protein.

3. The protein or polypeptide as claimed in claim 1, characterized in that it consists of the amino acids corresponding to positions 14 to 1193 of the amino acid sequence of the S protein.

4. The isolated protein or polypeptide as claimed in claim 1, characterized in that it consists of the amino acids corresponding to positions 475 to 1193 of the amino acid sequence of the S protein.

5. A nucleic acid encoding a protein or a polypeptide as claimed in any one of claims 1 to 4.

6. The nucleic acid as claimed in claim 5, characterized in that it comprises the sequence encoding SEQ ID No: 5 or the sequence encoding SEQ ID No: 6.

7. A recombinant expression vector, characterized in that it encodes a protein or a polypeptide as claimed in any one of claims 1 to 4.

8. The recombinant expression vector as claimed in claim 7, characterized in that it is chosen from the vectors contained in the following bacterial strains, deposited at the Collection Nationale de Cultures de Microorganismes (CNCM), 25 rue du Docteur Roux, 75724 Paris Cedex 15:

a) strain No. I-3118, deposited on Oct. 23, 2003,

b) strain No. I-3019, deposited on May 12, 2003,

c) strain No. I-3020, deposited on May 12, 2003,

d) strain No. I-3059, deposited on Jun. 20, 2003,

e) strain No. I-3323, deposited on Nov. 22, 2004,

f) strain No. I-3324, deposited on Nov. 22, 2004,

g) strain No. I-3326, deposited on Dec. 1, 2004,

h) strain No. I-3327, deposited on Dec. 1, 2004,

i) strain No. I-3332, deposited on Dec. 1, 2004,

j) strain No. I-3333, deposited on Dec. 1, 2004,

k) strain No. I-3334, deposited on Dec. 1, 2004,

l) strain No. I-3335, deposited on Dec. 1, 2004,

m) strain No. I-3336, deposited on Dec. 1, 2004,

n) strain No. I-3337, deposited on Dec. 1, 2004,

o) strain No. I-3338, deposited on Dec. 2, 2004,

p) strain No. I-3339, deposited on Dec. 2, 2004,

q) strain No. I-3340, deposited on Dec. 2, 2004, and

r) strain No. I-3341, deposited on Dec. 2, 2004.

9. A nucleic acid containing a synthetic gene allowing optimized expression of the S protein in eukaryotic cells, characterized in that it possesses the sequence SEQ ID No: 140.

10. An expression vector containing a nucleic acid as claimed in claim 9, characterized in that it is contained in the bacterial strain deposited at the CNCM, on Dec. 1, 2004, under the No. I-3333.

11. The expression vector as claimed in claim 7 or claim 9, characterized in that it is a viral vector, in the form of a viral particle or in the form of a recombinant genome.

12. The vector as claimed in claim 11, characterized in that it is a recombinant viral particle or a recombinant viral

genome capable of being obtained by transfecting a plasmid according to paragraphs g), h) or k) to r) of claim 8, into an appropriate cellular system.

13. A lentiviral vector encoding a polypeptide as claimed in any one of claims 1 to 4.

14. A recombinant measles virus encoding a polypeptide as claimed in any one of claims 1 to 4.

15. A recombinant vaccinia virus encoding a polypeptide as claimed in any one of claims 1 to 4.

16. The use of a vector according to paragraphs d) to p) of claim 8, or of a vector as claimed in claim 10, for the production, in a eukaryotic system, of the SARS-associated coronavirus S protein or of a fragment of this protein.

17. A method for producing the S protein in a eukaryotic system, comprising a step of transfecting eukaryotic cells in culture with a vector chosen from the vectors contained in the bacterial strains mentioned in paragraphs d) to p) of claim 8, or in claim 10.

18. A genetically modified eukaryotic cell expressing a protein or a polypeptide as claimed in any one of claims 1 to 4.

19. The cell as claimed in claim 18, capable of being obtained by transfection with any one of the vectors mentioned in paragraphs k) to n) of claim 8.

20. The cell as claimed in claim 19, characterized in that it is the cell FRhK4-Ssol-30, deposited at the CNCM on Nov. 22, 2004, under the No. I-3325.

21. A monoclonal antibody recognizing the native S protein of a SARS-associated coronavirus.

22. The use of a protein or a polypeptide as claimed in any one of claims 1 to 4, or of an antibody as claimed in claim 21, for detecting a SARS-associated coronavirus infection, from a biological sample.

23. A method for detecting a SARS-associated coronavirus, from a biological sample, characterized in that the detection is carried out by ELISA using the recombinant S protein or its ectodomain, or a fragment of its ectodomain, expressed in a eukaryotic system.

24. The method of detection as claimed in claim 23, additionally comprising a step of detection by ELISA using the recombinant N protein.

25. The method as claimed in claim 23 or 24, characterized in that it is a double epitope ELISA method, and in that the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.

26. An immune complex formed of a monoclonal antibody or antibody fragment as claimed in claim 21, and of a SARS-associated coronavirus protein or peptide

27. An immune complex formed of a protein or a polypeptide as claimed in any one of claims 1 to 4, and of an antibody directed specifically against an epitope of the SARS-associated coronavirus.

28. A SARS-associated coronavirus detection kit or box, characterized in that it comprises at least one reagent selected from the group consisting of: a protein or polypeptide as claimed in any one of claims 1 to 4, a nucleic acid as claimed in either of claims **5** and **6**, a cell as claimed in any one of claims 18 to 20, or an antibody as claimed in claim 21.

29. An immunogenic and/or vaccine composition, characterized in that it comprises a recombinant protein or polypeptide as claimed in any one of claims 1 to 4, obtained in a eukaryotic expression system.

30. An immunogenic and/or vaccine composition, characterized in that it comprises a recombinant vector or virus as claimed in any one of claims **7**, **8**, and **10** to **15**.

31. A nucleic acid insert of viral origin, characterized in that it is contained in any one of the strains mentioned in paragraphs a) to h) and k) to r) of claim 8.

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